(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018627 A3

(51) International Patent Classification[7]: C12N 7/00

(21) International Application Number:
PCT/US2003/026145

(22) International Filing Date: 21 August 2003 (21.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/405,182	22 August 2002 (22.08.2002)	US
60/455,234	17 March 2003 (17.03.2003)	US
60/455,312	17 March 2003 (17.03.2003)	US
60/458,825	28 March 2003 (28.03.2003)	US

(71) Applicant *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BETT, Andrew, J.** [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CHASTAIN, Michael** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SANDIG, Volker** [DE/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **EMINI, Emilio, A.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SHIVER, John, W.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CASIMIRO, Danilo, R.** [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **KASLOW, David, C.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **MORSY, Manal** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
2 September 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/018627 A3

(54) Title: METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY

(57) Abstract: Various methods for propagating and rescuing multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line are disclosed. Typically, replication-defective adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The disclosed methods offer the ability to propagate vectors derived from multiple adenoviral serotypes in a single production cell line which expresses E1 proteins from a single serotype. Propagation in this manner is accomplished by providing all or a portion of an E4 region *in cis* within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the E1 gene product(s) of the complementing cell line. Interaction between the expressed E1 of the cell line and the heterologous E4 of the replication-defective adenoviral vectors enables their propagation and rescue. The invention bypasses a need in the art to customize specific cell lines to the serotype or subgroup of the adenoviral vector being propagated and enables one to easily and rapidly develop alternative adenoviral serotypes as gene delivery vectors for use as vaccines or as a critical component in gene therapy.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/26145

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 7/00
US CL : 435/235.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/5, 235.1; 424/93.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 5,837,511 A (FALCK-PEDERSEN et al.) 17 November 1998 (17:11.1998), column 7, lines 47-55, 62-67, column 8, lines 6-18, column 9, lines 11-15, 54-57, column 12, lines 35-67, column 16, lines 23-49	1, 2, 4-10, 12, 13, 17-21, 24, 27-37, 43-50, 56-65, 71-78 ——— 3, 11, 14-16, 22, 23, 25, 26, 38-42, 51-55, 66-70, 79-83
Y	US 6,200,798 B1 (YEH et al.) 13 March 2001 (13.03.2001), column 5, lines 20-30	3, 14-16, 22, 23, 25, 26, 38-42, 51-55, 66-70, 79-83
Y	US 6,391,612 B1 (BRUDER et al.) 21 May 2002 (21.05.2002), column 5, lines 60-67, column 6, lines 1-10	11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2004 (19.03.2004)	12 JUL 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence for Tim Brown Telephone No. (571) 272-0773

Form PCT/ISA/210 (second sheet) (July 1998)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018627 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US2003/026145

(22) International Filing Date: 21 August 2003 (21.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/405,182	22 August 2002 (22.08.2002)	US
60/455,234	17 March 2003 (17.03.2003)	US
60/455,312	17 March 2003 (17.03.2003)	US
60/458,825	28 March 2003 (28.03.2003)	US

(71) Applicant *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

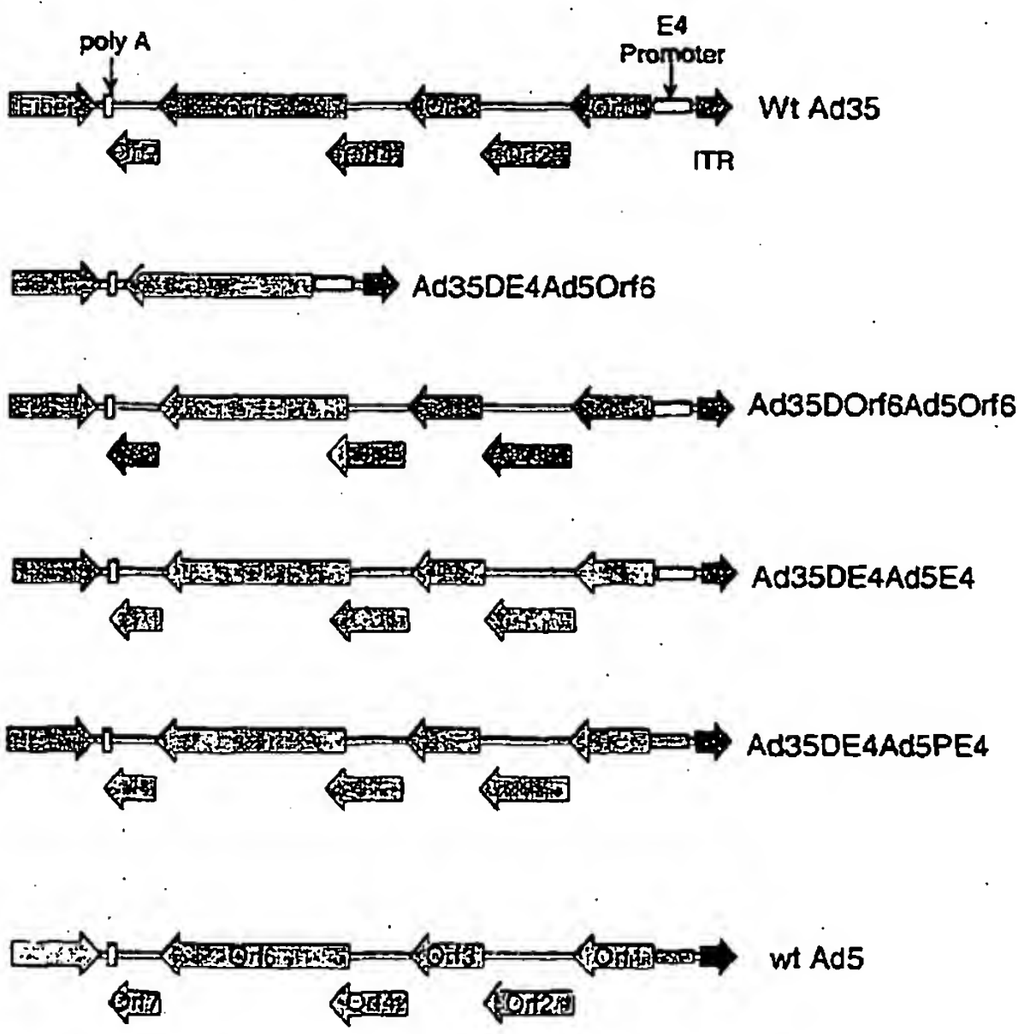
(75) Inventors/Applicants *(for US only)*: **BETT, Andrew, J.** [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CHASTAIN, Michael** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SANDIG, Volker** [DE/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **EMINI, Emilio, A.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SHIVER, John, W.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CASIMIRO, Danilo, R.** [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **KASLOW, David, C.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **MORSY, Manal** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY



(57) Abstract: Various methods for propagating and rescuing multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line are disclosed. Typically, replication-defective adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The disclosed methods offer the ability to propagate vectors derived from multiple adenoviral serotypes in a single production cell line which expresses E1 proteins from a single serotype. Propagation in this manner is accomplished by providing all or a portion of an E4 region *in cis* within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the E1 gene product(s) of the complementing cell line. Interaction between the expressed E1 of the cell line and the heterologous E4 of the replication-defective adenoviral vectors enables their propagation and rescue. The invention bypasses a need in the art to customize specific cell lines to the serotype or subgroup of the adenoviral vector being propagated and enables one to easily and rapidly develop alternative adenoviral serotypes as gene delivery vectors for use as vaccines or as a critical component in gene therapy.

WO 2004/018627 A2

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

METHODS FOR PROPAGATING ADENOVIRUS AND VIRUS PRODUCED THEREBY

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of application serial nos. 60/458,825, filed March 28, 2003; 60/455,312, filed March 17, 2003; 60/455,234, filed March 17, 2003; and 60/405,182, filed August 22, 2002.

FIELD OF THE INVENTION

The present invention concerns various methods to propagate and rescue multiple serotypes of replication-defective adenovirus in a single adenoviral E1-complementing cell line. Typically, replication-defective adenovirus vectors propagate only in cell lines which express E1 proteins of the same serotype or subgroup as the vector. The methods disclosed herein offer the ability to propagate vectors derived from multiple serotypes in a single cell line expressing E1 proteins from a single serotype. Such propagation of a wide range of vectors in one cell line is accomplished by providing all or a portion of an E4 region *in cis* within the genome of the replication-defective adenovirus. The added E4 region or portion thereof is cloned from a virus of the same or highly similar serotype as that of the E1 gene product(s) of the complementing cell line. Interaction between the E1 gene products of the cell line and the heterologous E4 gene products of the replication-defective adenoviral vector enables the propagation and rescue of the recombinant replication-defective adenovirus vectors. The invention, therefore, bypasses an existing need in the art to customize complementing cell lines to the specific serotype or subgroup of the adenoviral vector being propagated or, alternatively, to have to transfect a cell line with an E4 region and then regulate the expression *in trans* of the E4 region within the E1 complementing cell line.

BACKGROUND OF THE INVENTION

Beginning with the first human adenoviruses (Ads) isolated over four decades ago (Rowe *et al.*, *Proc. Soc. Exp. Biol. Med.*, *84*:570-579, 1953), over 100 distinct serotypes of adenovirus have been isolated which infect various mammalian species, 51 of which are of human origin (Straus, Adenovirus infections in humans. In *The Adenoviruses*. 451-498, 1984; Hierholzer *et al.*, *J. Infect. Dis.*, *158*: 804-813, 1988; Schnurr and Dondero, *Intervirology.*, *36*: 79-83, 1993; Jong *et al.*, *J Clin Microbiol.*, *37*:3940-3945:1999). The human serotypes have been categorised into six subgenera (A-F) based on a number of biological, chemical, immunological and structural criteria; criteria which include hemagglutination properties of rat

and rhesus monkey erythrocytes, DNA homology, restriction enzyme cleavage patterns, percentage of G+C content and oncogenicity (Straus, Adenovirus infections in humans. In *The Adenoviruses*. 451-498, 1984; Horwitz, Adenoviridae and their replication, *In Virology*: 1679-172, 1990).

Deletion of an essential E1 region common to the various adenovirus serotypes has enabled the use of adenovirus vectors as gene transfer vectors for vaccine and gene therapy purposes. Resultant replication-defective vectors are propagated in cell lines that provide the deleted E1 gene products *in trans*. Supplementation of the essential E1 gene products *in trans* in this manner works well when the E1 gene products are from the same or a highly similar serotype. As such, E1-deleted group C serotypes (Ad1, Ad2, Ad5 and Ad6) grow well in 293 or PER.C6 cells which contain and express the Ad5 E1 region. In contrast, E1-deleted serotypes other than group C, for example those from subgroups A, B, D, E, and F (*e.g.*, Ad3, Ad4, and Ad7 to Ad51), do not replicate efficiently in 293 or PER.C6 cells. The Ad5 E1 sequences in 293 and PER.C6 cells do not fully complement the replication of these alternative serotypes. This presents a challenge due to the fact that the most characterized and studied complementing cell lines available for growth and propagation of adenovirus are based on E1 sequence from adenovirus serotype 5.

This inability to fully complement the replication of serotypes other than group C adenovirus in Ad5 E1 complementing cell lines has been attributed to the inability of Ad5 (group C) E1b 55K gene product to functionally interact with the E4 gene products of non-group C serotypes. While the interaction is conserved within members of the same subgroup, it is not well conserved between subgroups.

Hence, cell lines expressing both Ad5 E1 and ORF6 were generated and proved useful in complementing alternative adenovirus serotypes; *see, e.g.,* Abrahamsen *et al.*, 1997 *J. Virol.* 8946-8951. Such incorporation of E4 (or ORF6) into Ad 5 complementing cell lines as was done in Abrahamsen *et al.*, *supra*, is known.

U.S. Patent No. 5,849,561 discloses complementation of an E1-deleted non-group C adenovirus vector in an Ad5-E1 complementing cell line which also expresses portions of the Ad5-E4 gene.

U.S. Patent No. 6,127,175, issued to Vigne, *et al.*, discloses a stably transfected mammalian cell line which expresses a portion of the E4 region of adenovirus, preferably ORF6 or ORF6/7. Such a cell line is useful for complementation of recombinant Ad genomes deficient in the E4 region.

European Application EP 1 054 064 A1 discloses recombinant, replication deficient adenovirus 35 (Ad35) vectors and cell lines which complement *in trans* the growth of

these vectors. A cell line which expresses Ad5E1A and E2A genes (PER.C6) was shown to complement an Ad35-E1 deleted vector upon co-expression of Ad35-E1B proteins.

U.S. Patent No. 6,270,996, issued to Wilson, *et al.*, discloses E1/E4 deleted adenovirus vectors and E1/E4(ORF6) cell lines which complement *in trans* virus growth without resulting in cell toxicity.

U.S. Patent No. 6,202,060, issued to Mehtali, *et al.*, discloses adenoviral vectors wherein portions of the early genes are under control of an inducible promoter. The '060 patent also discloses complementing cell lines which may be used in tandem with these Ad vectors.

The generation of serotype-specific cell lines providing a complementing serotype-specific E1 gene product(s) *in trans* is known as well.

Although Ad5-based vectors have been used extensively in a number of gene therapy trials, there may be limitations on the use of Ad5 and other group C adenoviral vectors due to preexisting immunity in the general population due to natural infection. Ad5 and other group C members tend to be among the most seroprevalent serotypes. Immunity to existing vectors may develop as a result of exposure to the vector during treatment. These types of preexisting or developed immunity to seroprevalent gene delivery vectors may limit the effectiveness of gene therapy or vaccination efforts. Alternative adenovirus serotypes, thus, constitute very important targets in the pursuit of gene delivery systems capable of evading the host immune response.

There remains both a practical and commercial need for an adenovirus-based vaccine and/or gene therapy delivery system which allows for the production of multiple serotype recombinant adenovirus vectors in a single source complementing mammalian cell line. The present invention addresses and overcomes this deficiency in the art by disclosing novel methods for propagating multiple serotype recombinant Ad vectors in a single complementing cell line where the required serotype-specific sequences are provided *in cis*.

SUMMARY OF THE INVENTION

The present invention relates to an enhanced means for propagating replication-defective adenovirus in an E1-complementing cell line(s) where the E1 gene product(s) being expressed is not native to the adenovirus being propagated. The method is based on Applicants' finding that supply, *in cis*, of a nucleic acid sequence encoding all or a portion of a heterologous adenoviral E4 region which is native to a virus of the same or highly similar serotype as the E1 gene product(s) of the complementing cell line enables the growth of adenoviral vectors of varying serotype in any single complementing cell line, despite the fact the cell line is not customized for the particular serotype of vector being propagated. This is of particular

importance given that existing and settled adenoviral E1-complementing cell lines (such as PER.C6™ and 293) are based on one of the most prominent adenovirus serotypes (Ad5) and are not suited for the large-scale propagation and rescue of alternative serotypes.

The basic steps involved in the propagation of adenoviral vectors in accordance with the methods of the instant invention are as follows: First, all or a portion of a heterologous adenoviral E4 region comprising nucleic acid sequence encoding at least open reading frame 6 (ORF6) is inserted into a replication-defective adenoviral vector. By "heterologous", Applicants mean that the nucleic acid sequence is not native to the viral vector being propagated, *i.e.*, not normally present within a virus of the same or highly similar serotype. As will be described, the adenoviral E4 region or portion thereof can be either a nucleic acid sequence encoding ORF 6 or any larger portion of the E4 region, and includes nucleic acid comprising the complete E4 region with E4 promoter. The region into which the nucleic acid is incorporated is not limited, *i.e.*, the insertion can be made into the complete E4 region with E4 promoter or into a smaller portion narrowing into the ORF6 region. Alternatively, the heterologous E4 region or portion thereof can be inserted into different areas of the genome such as the E1 or E3 regions. Further, the native E4 region or portion thereof can be deleted and replaced, or left intact. This is not deemed a critical element of the instant invention. What is a critical element is that the heterologous E4 region or portion thereof being inserted is native to a virus of the same or highly similar serotype as the E1 gene product(s) expressed by the complementing cell line.

Following the modification of the adenoviral vector of interest, the recombinant adenovirus is then introduced into an adenoviral E1-complementing cell line and allowed to propagate. The adenovirus is subsequently harvested and rescued from the complementing cell line.

The resultant virus can be studied and used in various gene therapy and vaccine efforts. The virus, therefore, forms an important aspect of the instant invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates a transcription map for adenovirus serotype 5. The linear genome is divided into 100 map units as well as into r- and l- strands which designate the direction of transcription. Early transcription units are designated with an E and are active prior to viral DNA replication. Late transcription units are designated with and L and are active primarily after DNA replication. Promoters are represented as brackets and polyadenylation sites as arrowheads. The tripartite leader is designated 1, 2, and 3.

FIGURES 2A-1 through 2A-10 illustrate the nucleic acid sequence for the wild-type adenovirus 35 (SEQ ID NO: 1) utilized in the Examples.

FIGURE 3 illustrates the homologous recombination scheme utilized to recover pAd35ΔE1.

FIGURE 4 illustrates the various configurations of the E4 regions (or portions) within the alternative serotype recombinants.

FIGURE 5 illustrates the homologous recombination scheme utilized to recover pAd35ΔE1ΔE4Ad5Orf6.

FIGURE 6 illustrates the nucleic acid sequence encoding the gag expression cassette (SEQ ID NO: 2). The various regions of the figure are as follows: (1) a first underlined segment of nucleic acid sequence encoding the immediate early gene promoter region from human cytomegalovirus; (2) a first segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; (3) a region in caps which contains the coding sequence of HIV-1 gag; (4) a second segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; and (5) a second underlined segment, this segment containing nucleic acid sequence encoding a bovine growth hormone polyadenylation signal sequence.

FIGURE 7 illustrates the nucleic acid sequence encoding the SEAP expression cassette (SEQ ID NO: 3). The various regions of the figure are as follows: (1) a first underlined segment of nucleic acid sequence encoding the immediate early gene promoter region from human cytomegalovirus; (2) a first segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; (3) a region in caps which contains the coding sequence of the human placental SEAP gene; (4) a second segment of lowercase letters which is not underlined, which segment of DNA contains a convenient restriction enzyme site; and (5) a second underlined segment, this segment containing nucleic acid sequence encoding a bovine growth hormone polyadenylation signal sequence.

FIGURE 8 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^10 vp doses of Ad35 vectors. This experiment was designed to address any effects of E3 deletion. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Shown are geometric means for each cohort of 5 mice.

FIGURE 9 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^10 vp doses of Ad35 vectors. This experiment was designed to address any effects of Ad5 sequence insertion into the Ad35 genome. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Two extra cohorts received 10^10 vp and 10^9 vp of Ad5 vector. Shown are geometric means for each cohort of 5 mice.

FIGURES 10A-B illustrate *in vivo* SEAP expression using MRKAd5-based (A) and Ad35ΔE1ΔE4Ad5Orf6-based (B) vector in rhesus macaques. Shown are the serum antigen

levels for individual monkeys following a single intramuscular (i.m.) injection of 10^11 vp MRKAd5SEAP (filled circles), 10^9 vp MRKAd5SEAP (open boxes) or 10^11 vp Ad35ΔE1SEAPΔE4Ad5Orf6.

FIGURE 11 illustrates *in vivo* SEAP expression in African green monkeys using Ad5- and Ad35-based vectors. Shown are the antigen levels for each animal in serum samples collected two days after the treatment.

FIGURE 12 illustrates the homologous recombination scheme utilized to recover pAd24ΔE1.

FIGURE 13 illustrates the homologous recombination scheme utilized to recover pAd24ΔE1Ad5Orf6.

FIGURE 14 illustrates the configuration of E4 regions in the Ad24 recombinants generated.

FIGURE 15 illustrates the growth kinetics of the Ad24-based vectors in PER.C6 cells.

FIGURES 16A-1 through 16A-10 illustrate the nucleic acid sequence for wild-type adenovirus serotype 24 (SEQ ID NO: 5). The ATCC product number for Ad24 is VR-259.

FIGURE 17 illustrates, in tabular format, gag-specific T cell responses in monkeys immunized with MRKAd5-HIVgag and Ad24 HIV vectors. Shown are the numbers of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag peptide pool. The pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

FIGURE 18 illustrates, in tabular format, the characterization of the gag-specific T cells in monkeys immunized with 10^11 vp of MRKAd5-HIV1gag and Ad24ΔE1gagΔOrf6Ad5Orf6. Shown are the percentages of CD3+ T cells that are either gag-specific CD4+ or gag-specific CD8+ cells. These values were corrected for mock values (<0.03%).

FIGURE 19 illustrates individual anti-p24 titers (in mMU/mL) in macaques immunized with gag-expressing adenovirus vectors.

FIGURE 20 illustrates *in vivo* expression of SEAP in C3H/HeN mice using 10^10 vp doses of Ad24 vectors. The vectors were injected intramuscularly and the levels of SEAP expression were determined from the serum samples. Two extra cohorts received 10^10 vp and 10^9 vp of Ad5 vector. Shown are geometric means for each cohort of 5 mice.

FIGURE 21 illustrates *in vivo* SEAP expression using MRKAd5 and Ad24 vectors in rhesus macaques. Shown are the geometric means of the SEAP levels for cohorts of 3 monkeys. In bars are the standard errors of the geometric means.

FIGURE 22 illustrates a homologous recombination scheme to be utilized to recover pAd24ΔE1ΔE4Ad5Orf6.

FIGURE 23 illustrates gag-specific T cell responses in rhesus macaques immunized following a heterologous Ad5/Ad6 prime-Ad24 boost regimen. a: Mock, no peptide: gag, 20-mer peptide pool encompassing entire gag sequence; b: Peak response after 2 or 3 doses of the priming vaccine; c: 3 wks prior to boost; d: 4 wks after boost; e: ND, not determined.

FIGURE 24 illustrates, in tabular format, the percentages of $CD3^+$ T lymphocytes that are gag-specific $CD8^+$ cells or gag-specific $CD4^+$ cells determined after the Ad24 Boost Immunization (wk 60). Numbers reflect the percentages of circulating CD3+ lymphocytes that are either gag-specific CD4+ or gag-specific CD8+ cells. Mock values (equal to or less than 0.01%) have been subtracted.

FIGURE 25 illustrates gag-specific T cell responses in rhesus macaques immunized following a heterologous Ad 24 prime-Ad5 boost regimen. a: Mock, no peptide: gag, 20-mer peptide pool encompassing entire gag sequence; b: Peak response after 2 doses of the priming vaccine; c: Wk 24; d: 4 wks after boost; e: ND, not determined.

FIGURE 26 illustrates the homologous recombination scheme utilized to recover pAd34ΔE1ΔE4Ad5Orf6.

FIGURE 27 illustrates the homologous recombination scheme utilized to recover pMRKAd34ΔE1ΔE4Ad5Orf6.

FIGURES 28A-1 to 28A-9 illustrate a nucleic acid sequence for wild-type adenovirus serotype 34 (SEQ ID NO: 12). The ATCC product number for Ad34 is VR-716.

FIGURE 29 illustrates the time course of SEAP expression using MRKAd5 and Ad34 vectors in rhesus macaques. Data represent cohort geometric means.

FIGURE 30 illustrates, in tabular format, T cell responses induced using MRKAd5 and Ad34 vectors expressing HIV-1 gag. Data are expressed in numbers of spot-forming cells per million PBMC (SFC/10^6 PBMC). "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 31 illustrates, in tabular format, the levels of CD4+ and CD8+ Gag-specific T cells in Ad34-immunized macaques at week 12. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 32 illustrates, in tabular format, T cell responses induced using a heterologous Ad34 prime/Ad35 boost regimen in macaques. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

FIGURE 33 illustrates, in tabular format, the levels of CD4+ and CD8+ Gag-specific T cells in Ad34 primed/Ad35 boosted macaques at week 28. "a" refers to a 20-mer peptide pool with 10-aa overlap and encompassing the entire HIV-1 CAM1 gag.

DETAILED DESCRIPTION OF THE INVENTION

The present invention details an efficient strategy for the propagation and rescue of alternative adenoviral serotypes utilizing available adenovirus production cell lines, nullifying the need to customize available cell lines for a specific serotype of interest. This is enabled by the incorporation of a critical E4 region into the adenovirus to be propagated.

The critical E4 region in the instant invention comprises, in the minimum, nucleic acid sequence encoding E4 ORF6 and can comprise the entire region of E4, inclusive of the promoter region. An important characteristic of the imported E4 region is that it is native to a virus of the same or highly similar serotype as the E1 gene product(s) (particularly E1B 55K) of the E1-complementing cell line, but heterologous to (*i.e.,* non-native to a virus of the same serotype as) the adenoviral vector being propagated. As will be detailed below, the heterologous E4 region or portion thereof can be varied and can be inserted into the vector backbone at numerous locations.

The heterologous E4 region or portion thereof can, for instance, be a nucleic acid sequence encoding the entire open reading frame of the non-native E4. This segment of nucleic acid sequence can, in turn, be incorporated into the "native" entire E4 open reading frame of the recipient virus. In such an embodiment, the promoter native to the adenoviral vector would drive the expression of the non-native E4 region within the recombinant replication-defective adenoviral vector. Alternatively, the nucleic acid sequence encoding the entire open reading frame can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

In another embodiment, the heterologous E4 region comprises a nucleic acid sequence encoding the entire open reading frame of E4 and includes a non-native E4 promoter. In this type of embodiment, the E4 region can be inserted into the location of the combined native E4 and E4 promoter region. The non-native E4 region in this embodiment would be driven by expression of the non-native E4 promoter. Alternatively, the nucleic acid sequence encoding the entire open reading frame and the non-native E4 promoter can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

An alternative and further embodiment exists wherein the heterologous E4 region or portion thereof comprises nucleic acid sequence encoding a partial E4 region comprising ORF6 (one aspect of which is a region solely encoding ORF6). In this particular aspect of the invention, the heterologous non-native E4 protein can, in certain embodiments, replace the non-native ORF6 region or the entire E4-encoding region of the native virus. In the latter situation, the promoter driving expression of the non-native ORF6 can either be the native E4 promoter or a heterologous, non-native promoter operatively linked to the non-native ORF6, while in the latter, the expression of the non-native ORF6 would generally be driven by the native E4 promoter. Alternatively, the nucleic acid sequence encoding a partial E4 region comprising ORF 6 can be inserted into a different region of the adenoviral vector genome, such as for example the E1 or E3 regions. In this latter embodiment, the native E4 region or portion thereof can be deleted or left intact.

As one of skill in the art can appreciate, there are various ways in which one can envision the supply of a heterologous E4 nucleic acid sequence *in cis* to an adenoviral vector and thereby enable its growth based on Applicants' novel findings herein. Moreover, as one of skill in the art can appreciate, either native or non-native promoters can be utilized to drive expression of the heterologous E4 region or portion thereof.

Adenovirus pre-plasmids (plasmids comprising the genome of the replication-defective adenovirus with desired deletions and insertions) can be generated by homologous recombination using adenovirus backbones and an appropriate shuttle vector (designed to target-in specific deletions and incorporate desired restriction sites into the resultant plasmid). Shuttle vectors of use in this process can be generated using general methods widely understood and appreciated in the art, *e.g.*, PCR of the adenoviral terminal ends taking into account the desired deletions, and the sequential cloning of the respective segments into an appropriate cloning plasmid. The adenoviral pre-plasmid can then be digested and transfected into the complementing cell line via calcium phosphate co-precipitation or other suitable means. Virus replication and amplification then occurs, a phenomenon made evident by notable cytopathic effect. Infected cells and media are then harvested after viral replication is complete (generally, 7-10 days post-transfection).

It is to be noted that various alternative adenoviral serotypes can be developed in accordance with the disclosed methods and, particularly, alternative adenoviral serotype vectors that were previously unable to be propagated or very inefficiently propagated utilizing existing adenoviral production cell lines based on subgroup C complementing E1 sequence. The various adenoviral vectors that can be developed in accordance with the instant methods include adenoviral vectors of subgroups A-F (for instance, serotypes of subgroups A, B (*e.g.*, serotypes

11, 14, 16, 21, 34 and 35), C (*e.g.*, serotypes 2 and 5), D (*e.g.*, serotypes 24, 26 and 36), E (*e.g.*, serotype 4) and F.

In preferred embodiments, the various non-group C family members can be developed with heterologous E4 supplied from a subgroup C member such as adenovirus serotype 5. Particular embodiments of the instant invention utilize a development scheme wherein the heterologous E4 protein is derived from a wildtype adenovirus serotype 5 sequence; *see, e.g.*, a viral sequence which has been deposited with the American Type Culture Collection ("ATCC") under ATCC Deposit No. VR-5 (for which a transcription map can be found in Figure 1). A particular example of this type of embodiment is wherein an adenovirus of subgroup B (or any non-C subgroup) comprising heterologous E4 proteins *in cis* from Ad5 is propagated in Ad5 E1-complementing cell lines, for instance, PER.C6™ or 293. Applicants have, in fact, successfully propagated E1- serotypes 10, 24, 34, and 35 via use of this particular embodiment.

One of skill in the art can readily identify alternative adenovirus serotypes (*e.g.*, alternative serotypes of subgroups A, B (*e.g.*, serotypes 11, 14, 16, 21, 34 and 35), C, (*e.g.*, serotypes 2 and 5), D (*e.g.*, serotypes 24, 26 and 36), E (*e.g.*, serotype 4) and F) for the supply of the heterologous E4 protein. As long as the heterologous E4 region (or portion thereof comprising ORF6) of the vector is native to a virus of the same or highly similar serotype as the E1 region of the complementing cell line, the methods of the instant invention are widely applicable to the propagation and rescue of adenovirus of all serotypes. In light of the present disclosure, one can readily envision, for instance, how a complementing cell line based on a non-subgroup C adenovirus (*e.g.*, the Ad35 cell line of EP 1 054 064 A1) can be utilized to propagate a virus of an adenoviral vector of subgroup C (*e.g.*, adenovirus serotype 5) provided that the appropriate nucleic acid sequence encoding an E4 protein provided *in cis* is native to a virus of the same or highly similar serotype as that of the E1 expressed by the complementing cell line (*i.e.*, an Ad35 E4 protein).

Complementing cell lines of use in the instant invention are available in the art and are not limited to any specific type. The critical feature, again, is that the heterologous segment of E4-encoding nucleic acid sequence provided *in cis* to the replication-defective vector being propagated be native to a virus of the same or highly similar serotype as the E1 expressed by the complementing cell line. One aspect of the instant invention employs E1-complementing cell lines wherein the expressed E1 is of serotype 5; *e.g.*, PER.C6™ and 293 cell lines. Both these cell lines express the adenoviral E1 gene product. PER.C6™ is described in Fallaux *et al.*, 1998 *Human Gene Therapy* 9:1909-1917, hereby incorporated by reference. 293 cell lines are described in Graham *et al.*, 1977 *J. Gen. Virol.* 36:59-72, hereby incorporated by reference.

Another aspect of the instant invention are the adenoviral vectors of any serotype falling with adenoviral subgroups A, B, C, D, E and F (for instance, alternative serotypes of subgroups A, B (*e.g.*, serotypes 11, 14, 16, 21, 34 and 35), C (*e.g.*, serotype 2), D (*e.g.*, serotypes 24, 26 and 36), E (*e.g.*, serotype 4) and F) which are modified to contain a non-native E4-encoding nucleic acid sequence *in cis* which comprises, in whole or in part, nucleic acid sequence encoding open reading frame 6 (ORF6). Virus in accordance with this description can be propagated in accordance with the above-described methods and rescued using any suitable means known in the art.

Another aspect of the instant invention is a vector in accordance with the instant invention which comprises a heterologous passenger gene in addition to that of the heterologous E4 nucleic acid sequence. In specific embodiments, the passenger gene encodes an antigen.

As one of ordinary skill in the art will appreciate, the instant methods are not limited by the heterologous gene that can be incorporated. The instant invention relates generally to a means by which to propagate multiple serotypes of adenovirus in a single complementing cell line and the recombinant virus that make the process possible. In preferred embodiments, the passenger gene is incorporated into the E1 deletion. In alternatively preferred embodiments, the passenger gene is inserted in an E3-deleted region. The position of the passenger gene, as one of ordinary skill in the art will appreciate, can be varied according to the specific complementing cell utilized and the specific deletions present within the replication-defective adenovirus genome.

In specific embodiments the passenger gene can encode an HIV-1 antigen, and in more preferred embodiments selected from the group consisting of genes encoding HIV-1 gag, pol, nef and env. In alternative embodiments, the passenger gene can be a reporter gene, such as secreted alkaline phosphatase (SEAP).

The passenger gene preferably exists in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid sequence encoding a protein of interest; (b) a promoter operatively linked to the nucleic acid sequence encoding the protein; and (c) a transcription termination sequence. The transcriptional promoter of the adenoviral vector is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman *et al.*, 1991 *Nucl. Acids Res.* 19:3979-3986), which is hereby incorporated by reference), in certain embodiments without intronic sequences. Those skilled in the art, however, will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters

may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

The promoter may comprise a regulatable sequence such as the Tet operator sequence. This is extremely useful, for example, in cases where the gene products are affecting a result other than that desired and repression is sought.

Transcription termination sequences can also be utilized within the gene expression cassettes. Preferred termination sequences are, for instance, the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:4).

Further embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Construction and Rescue

An E1- Ad35-based pre-adenovirus plasmid was constructed in order to determine whether an E1- Ad35 vector (a representative group B serotype) could be propagated in a group C E1-complementing cell line. The general strategy used to recover Ad35 as a bacterial plasmid is illustrated in Figure 3. Cotransformation of BJ5183 bacteria with purified wild-type Ad35 viral DNA and a second DNA fragment termed the Ad35 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome (*see* Figures 2A-1 to 2A-10) separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad5 457 to 3402 with a unique *Swa* I site located in the deletion. The Ad35 sequences in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which recombination can occur. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1. Pre-Adenovirus plasmid pAd35ΔE1 contains Ad35 sequences from 4 to 456 and bp 3403 to 34793.

To determine if pre-adenovirus plasmid pAd35ΔE1 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into a T-25 flask of PER.C6 cells using the calcium phosphate co-precipitation technique. *Pme* I digestion releases the viral genome from the plasmid sequences allowing viral replication to occur after entry into 293 cells. Viral cytopathic effect (CPE), indicating that virus replication and amplification is occurring, was never observed. Cells and media from the transfection were harvested at 14 days post transfection, freeze-thawed three times, clarified by centrifugation and used to infect new PER.C6 cells but no virus was ever amplified. Following multiple attempts, we have been unable to rescue and amplify pAd35ΔE1 in PER.C6 cells.

EXAMPLE 2

Insertion of Ad5 Orf 6 and Ad5 E4 into the Ad5 Genome

To refine the strategy of including Ad5 Orf6 in the genome of an alternative serotype so that propagation could take place in a Ad5/group C complementing cell line four additional strategies were developed. In the first strategy, the entire alternative serotype E4 region (not including the E4 promoter) was deleted and replaced with Ad5 Orf6. In the second strategy, just the alternative serotype Orf6 gene was deleted and replaced with Ad5 Orf6. In the third strategy, the entire alternative serotype E4 coding region (not including the E4 promoter) was deleted and replaced with the Ad5 E4 coding region (not including the Ad5 E4 promoter) and, in the final strategy, the entire alternative serotype E4 coding and promoter region was deleted and replaced with the Ad5 E4 promoter and coding region. The configuration of the E4 regions generated by the four strategies is diagramed in Figure 4. For each of these strategies the desired pre-Adenovirus plasmid was generated by bacterial recombination. Cotransformation of BJ 5183 bacteria with purified wild-type viral DNA and the appropriately constructed ITR cassette resulted in the circularization of the viral genome by homologous recombination. The construction of each pre-Ad plasmid, based on Ad35, is outlined below:

To construct pAd35ΔE1ΔE4Ad5Orf6 (An Ad35 pre-Ad plasmid containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31913 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 31913 and 34419 generating pNEBAd35-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a

bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31912 to 34418 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct, Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31913 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria (Figure 5). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5Orf6. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5Orf6 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31913 and bp 34419 to bp 34793 with Ad5Orf6 cloned between bp 31913 and bp 34419.

To construct pAd35ΔE1ΔOrf6Ad5Orf6 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 Orf6 substituted with Ad5 Orf6), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 32081 and bp 32990 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-10. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 32081 and 32990 generating pNEBAd35-10Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and a deletion of E4 Orf6 from Ad35 bp 32082 to 32989 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct, Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 32081 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔOrf6Ad5Orf6. Pre-Adenovirus plasmid pAd35ΔE1ΔOrf6Ad5Orf6 contains Ad35

sequences from bp 4 to 456; bp 3403 to bp 32081 and bp 32990 to bp 34793 with Ad5Orf6 cloned between bp 32081 and bp 32990.

To construct pAd35ΔE1ΔE4Ad5E4 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 substituted with Ad5 E4), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31838 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-7. Next the Ad5 E4 coding region was generated by PCR and cloned between Ad35 bp 31838 and 34419 generating pNEBAd35-7Ad5E4-2 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31839 to 34418 into which the Ad5 E4 coding region was introduced in an E4 parallel orientation. In this construct, the Ad5 E4 region is expressed using the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31838 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5E4. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5E4 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31838 and bp 34419 to bp 34793 with the Ad5 E4 coding region (Ad 5 bp 32914 to bp 35523) cloned between bp 31838 and bp 34419.

To construct pAd35ΔE1ΔE4Ad5PE4 (An Ad35 pre-Ad plasmid containing an E1 deletion and a deletion of E4 coding region and promoter substituted with Ad5 E4 coding region and promoter), an Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31838 and bp 34660 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-8. Next the Ad5 E4 promoter and coding region was generated by PCR and cloned between Ad35 bp 31838 and 34660 generating pNEBAd35-8Ad5E4PC (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication,

ampicillin resistance gene, and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31839 to 34659 into which the Ad5 E4 promoter and coding region was introduced in an E4 parallel orientation. In this construct, the Ad5 E4 region is expressed using the Ad5 E4 promoter. The Ad35 sequences (bp 31599 to 31838 and bp 3403 to 3886) in the ITR cassette provide regions of homology with the purified Ad35 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the recombinant Ad35 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd35ΔE1ΔE4Ad5PE4. Pre-Adenovirus plasmid pAd35ΔE1ΔE4Ad5PE4 contains Ad35 sequences from bp 4 to 456; bp 3403 to bp 31838 and bp 34660 to bp 34793 with the Ad5 E4 promoter and coding region (Ad 5 bp 32914 to bp 35826) cloned between bp 31838 and bp 34660.

EXAMPLE 3

Rescue of pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4 into Virus

In order to determine if pre-adenovirus plasmids pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmids were each digested with *Pme* I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique; Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc. *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring, was observed for all construct. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect aT-225 flasks of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then

digested with *Hind*III and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme*1/*Hind*III prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 4

Insertion of an Expression Cassette into pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4

In order to introduce a gag or SEAP expression cassette into the E1 region of the various Ad35 pre-Adenovirus plasmids described above (pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4 and pAd35ΔE1ΔE4Ad5PE4) bacterial recombination was again used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence (Figure 6), was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2 (a precursor to the Ad35 ITR cassettes described above), generating pNEBAd35CMVgagBGHpA. pNEBAd35-2 contains Ad35 sequences from the left end of the genome (bp 4 to 456 and bp 3403 to 3886) with a unique SwaI site between bp 456 and 3403 at the position of the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pNEBAd35-2. This cloning step resulted in the gag expression cassette being cloned into the E1 deletion between bp 456 and 3403 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad35 bp 4 to 456 and bp 3403 to 3886 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad35 pre-Ad plasmids (pAd35ΔE1ΔE4Ad5Orf6, pAd35ΔE1ΔOrf6Ad5Orf6, pAd35ΔE1ΔE4Ad5E4, pAd35ΔE1ΔE4Ad5PE4), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of corresponding Ad35 gag-containing pre-Adenovirus plasmids (pAd35ΔE1gagΔE4Ad5Orf6, pAd35ΔE1gagΔOrf6Ad5Orf6, pAd35ΔE1gagΔE4Ad5E4, and pAd35ΔE1gagΔE4Ad5PE4) by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad35 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence (Figure 7) was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2, generating pNEBAd35CMVSEAPBGHpA. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pNEBAd35-2. The transgene was then recombined into the various Ad35 backbones generating pAd35ΔE1SEAPΔE4Ad5Orf6, pAd35ΔE1SEAPΔOrf6Ad5Orf6, pAd35ΔE1SEAPΔE4Ad5E4, and pAd35ΔE1SEAPΔE4Ad5PE4 as described above for the gag transgene.
All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

EXAMPLE 5

In vivo Transgene Expression

A. Immunization

Female mice were between 4-10 weeks old. The total dose of each vaccine was suspended in 0.1 mL of buffer. The vectors were given to both quadriceps of each animals with a volume of 50 μL per quad and using 0.3-mL 28G1/2 insulin syringes (Becton-Dickinson, Franklin Lakes, NJ). The rhesus macaques and African green monkeys were between 2-5 kg in weight. For the primates, the total dose of each vaccine was suspended in 1 mL of buffer. The monkeys were anesthetized (ketamine/xylazine mixture) and the vaccines were delivered i.m. in 0.5-mL aliquots into two muscle sites using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Serum samples were collected at defined intervals and stored frozen until the assay date. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

Serum samples were analyzed for circulating SEAP levels using TROPIX phospha-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 μL aliquots of each serum were mixed with 45 μL of kit-supplied dilution buffer in a 96-well white DYNEX plate.

Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey or mouse serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the well for 30 minutes at 65 °C. Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorder using DYNEX luminometer. RLU readings are converted to ng/mL SEAP using a log-log regression analyses.

C. Rodent Results

In the first mouse experiment, cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^10 vp Ad35ΔE1SEAPΔE4Ad5Orf6; (2) 10^10 vp Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6; or (3) 10^10 vp Ad35ΔE1SEAP. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 8. Results indicate that (1) the Ad35 constructs are all capable of expressing the SEAP transgene and that (2) the introduction of Ad5Orf6 sequence where the deleted Ad35E4 was did not significantly affect the transgene expression relative to Ad35ΔE1SEAP. Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6 also yielded a similar expression profile as Ad35ΔE1SEAP. The levels of SEAP in the serum dropped after day 2 and were at background levels by day 12.

The second mouse experiment evaluates the effect of a full Ad5E4 replacement instead of an Ad5Orf6 substitution for the Ad35 E4 cassette. Here, cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^10 vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; (3) 10^10 vp Ad35ΔE1SEAPΔE4Ad5Orf6; (4) 10^10 vp Ad35ΔE1SEAPΔE4Ad5E4; or (5) 10^10 vp Ad35ΔE1SEAPΔE4Ad5PE4. The introduction of Ad5E4 or Ad5PE4 resulted in comparable if not, slightly improved expression levels compared to the vector with the Ad5Orf6 insertion (Figure 9). The peak levels for the Ad35 constructs are lower than those produced by Ad5SEAP (at least 10-fold).

D. Primate Results

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the following vectors: (1) 10^11 vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; or (3) 10^11 vp Ad35ΔE1SEAPΔE4Ad5Orf6. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results for the individual monkeys are shown in Figures 10A-B. Results indicate that the peak level of SEAP product produced by the alternative adenovirus serotype was lower than but were within 3-fold of that of MRKAd5SEAP at the same

high dose level of 10^11 vp. The levels observed from the Ad35 vector were about 50-fold higher than those observed using 10^9 vp of MRKAd5SEAP. The levels of SEAP in the serum dropped after day 10 and were close to background as early as day 15.

A separate experiment using African green monkeys was conducted to examine the effect of the additional E3 deletion or the full Ad5E4 substitution on in vivo gene expression. In here, cohorts of 2-3 African green macaques were given single intramuscular injections of one of the following vectors: (1) 10^11 vp MRKAd5-SEAP; (2) 10^10 vp MRKAd5-SEAP; (3) 10^9 vp MRKAd5-SEAP; (4) 10^10 vp Ad35ΔE1SEAPΔE4Ad5Orf6; (5) 10^10 vp Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6; or (6) 10^10 vp Ad35ΔE1SEAPΔE4Ad5E4. Results (Figure 11) indicate that the peak levels of SEAP product produced by Ad35ΔE1SEAPΔE3ΔE4Ad5Orf6 and Ad35ΔE1SEAPΔE4Ad5E4 were comparable if not, slightly improved compared to Ad35ΔE1SEAPΔE4Ad5Orf6.

EXAMPLE 6

In vivo Immunogenicity

A. Immunization

Cohorts of 3-6 animals were given intramuscular injections at wk 0 and wk 4 of either of the following constructs: (1) 10^11 vp MRKAd5-HIV1 gag; or (2) 10^11 vp of Ad35ΔE1gagΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson). Sera and peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. ELISPOT Assay

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μL of 2-4 x 10^5 peripheral blood mononuclear cells (PBMCs)

were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 µL of media or the gag peptide pool at 8 µg/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO_2 for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10^6 cell input.

C. Intracellular Cytokine Staining

To 1 ml of 2 x 10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 µg/mL. For gag-specific stimulation, 10 µL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 µL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO_2, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 µL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 µL anti-hCD8-PerCP, clone SK1 (Becton Dickinson, Franklin Lakes, NJ); and 20 µL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 µL 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 µg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both $CD4^+$ and $CD8^+$ populations, and for both mock and gag-peptide reaction tubes of a sample.

D. Results

PBMCs collected at regular 4-wk intervals were analyzed in an ELISPOT assay. Results (Table 1) indicate that the Ad35ΔE1gagΔE4Ad5Orf6 is able to induce in non-human primates significant levels of gag-specific T cells. After a single dose (wk 4), the Ad35-induced responses were about 5-fold lower than that of MRKAd5-HIV1 gag. After the second dose (wk

8), the responses between both cohorts were comparable; the differences became pronounced in the succeeding time points.

Table 1. Gag-specific T cell response in monkeys immunized with MRKAd5-HIV1gag and Ad35ΔE1gagΔE4Ad5Orf6. Shown is the number of spot-forming cells per million PBMC following incubation in the absence (mock) or presence of Gag H peptide pool. The H pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

Grp	Vaccine Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8		Wk 12		Wk 16	
			Mock	Gag H	Mock	Gag H	Mock	Gag H	Mock	Gag H	Mock	Gag H
1	MRKAd5-HIV1 gag 10^11 vp	00C018	1	5	13	1025	0	824	3	753	1	533
		00C034	0	4	5	219	5	404	0	491	1	350
		00C058	4	4	3	1086	0	440	0	439	0	599
2	Ad35ΔE1gagΔE4Ad5Orf6 10^11 vp	00D045	1	1	3	168	5	645	4	178	0	91
		00D067	1	4	5	89	0	103	0	76	0	19
		00D068	1	4	10	34	5	365	3	143	0	95
		00D054	3	15	10	195	0	501	3	350	0	124
		00D075	3	5	18	275	13	716	3	158	0	103
		00D073	14	26	1	241	3	485	3	278	0	148
3	Naive	00D087	1	1	3	3	8	54	3	5	3	1

Intracellular IFN-γ staining analyses of PBMC collected at wk 8 suggest that the Ad35-based vaccine is able to induce both HIV-specific CD4+ and CD8+ T cells (Table 2).

Table 2. Characterization of the gag-specific T cells in monkeys immunized with MRKAd5-HIV1gag and Ad35ΔE1gagΔE4Ad5Orf6. Shown are the percentages of CD3+ T cells that are either gag-specific CD4+ or gag-specific CD8+ cells. These values were corrected for mock values (<0.02%).

Grp	Vaccine Wk 0, Wk 4	Monkey ID	Wk 8	
			%CD4+CD3+	%CD8+CD3+
1	MRKAd5-HIV1 gag 10^11 vp	00C018	0.08	0.37
		00C034	0.09	0.06
		00C058	0.03	0.21
2	Ad35ΔE1gagΔE4Ad5Orf6 10^11 vp	00D045	0.06	0.08
		00D067	0.02	0.02
		00D068	0.15	0.02
		00D054	0.05	0.08
		00D075	0.08	0.05
		00D073	0.09	0.06

In a separate experiment, 3 different Ad35 constructs expressing HIV-1 gag were evaluated for their immunogenicity in macaques. Here, cohorts of 3 macaques were given immunizations at wk 0 and 4 of either of the following vectors: (1) 10^10 vp Ad35ΔE1gagΔE4Ad5Orf6; (2) 10^10

vp Ad35ΔE1gagΔE3ΔE4Ad5Orf6; or (3) 10^10 vp Ad35ΔE1gagΔE4Ad5E4. The levels of T cell immunity induced by all 3 vectors were comparable at this stage (Table 2), suggesting that the additional E3 deletion or full Ad5E4 substitution does not appear to impair the immunogenic properties of the vector.

Table 3. Gag-specific T cell response in monkeys immunized with several Ad35ΔE1ΔE4-based vectors. Shown is the number of spot-forming cells per million PBMC following incubation in the absence (mocK0 or presence of Gag H peptide pool. The H pool consisted of 20-aa peptide overlapping by 10 aa and encompassing the entire gag sequence.

Grp	Vaccine Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8	
			Mock	Gag H	Mock	Gag H	Mock	Gag H
1	Ad35ΔE1gagΔE4Ad5Orf6 10^10vp	00C047	4	1	0	20	0	189
		00C157	8	5	1	81	1	833
		00C078	3	1	0	46	4	349
2	Ad35ΔE1gagΔE3ΔE4Ad5Orf6 10^10vp	00C091	1	1	1	118	3	315
		00C122	3	0	0	31	1	138
		00D177	3	3	1	45	1	64
3	Ad35ΔE1gagΔE4Ad5E4 10^10vp	00D018	3	19	29	120	23	193
		00D046	8	5	1	21	10	143
		00D063	3	4	0	63	4	371
Naïve	none	00D363	0	5	ND	ND	0	0

EXAMPLE 7

Construction and Rescue of pAd24ΔE1.

An E1- Ad24-based pre-adenovirus plasmid was constructed in order to determine whether an E1- Ad24 vector (a representative group D serotype) could be propagated in an Ad5/group C E1-complementing cell line. Since at the time the vector construction was initiated the complete sequence of Ad24 (*see* Figures 16A-1 through 16A-10; subject of copending application serial no. 60/455, 312, filed March 17, 2003) was unknown we took advantage of some sequence homology between Ad24 and Ad17. The general strategy used to recover Ad24 as a bacterial plasmid is illustrated in Figure 12 and described below. Cotransformation of BJ5183 bacteria with purified wild-type Ad24 viral DNA and a second DNA fragment termed the Ad17 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The ITR cassette contains sequences from the right (bp 34469 to 35098) and left (bp 4 to 414 and bp 3373 to 4580) end of the Ad17 genome (Accession No. AF108105) separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad17

(bp 415 to 3372) with a unique *Swa* I site located in the deletion. The Ad17 sequences in the ITR cassette provide regions of homology with the purified Ad24 viral DNA in which recombination can occur. The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme* I) located at the end of the viral ITR's so that digestion will release the Ad24 genome from plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd24ΔE1. pAd24ΔE1 contains Ad17 sequences from bp 4 to 414 and from bp 3373 to 4580, Ad24 bp 4588 to 34529, and Ad17 bp 34469 to 35098 (bp numbers refer to the wt sequence for both Ad17 and Ad24). PAd24ΔE1 contains the coding sequences for all Ad24 virion structural proteins that constitute its serotype specificity. This approach can be used to circularize any group D serotype into plasmid form which has sufficient homology to Ad17.

To determine if pre-adenovirus plasmid pAd24ΔE1 could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into a 6 cm dish of 293 cells using the calcium phosphate co-precipitation technique. *Pme* I digestion releases the viral genome from the plasmid sequences allowing viral replication to occur after entry into 293 cells. Viral cytopathic effect (CPE), indicating that virus replication and amplification is occurring, was very slow to arise. Following multiple attempts, we were successful at rescuing and amplifying Ad24ΔE1 but the virus grew to lower titers and took more passages to amplify than a similar Ad5 based vector. In order to verify the genetic structure of the virus, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hin*dIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pme*1/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

EXAMPLE 8

<u>Insertion of Ad5 Orf 6 into the E1 region of Ad24</u>

In order to determine if the insertion of Ad5 E4 Orf6 into the Ad24 genome would allow more efficient propagation in a group C E1 complementing cell line we constructed an Ad24 based pre-adenovirus plasmid containing Ad5 Orf6 in the E1 region. In order to introduce Ad5 Orf6 in to the E1 region of pAd24ΔE1, bacterial recombination was used. An Ad5 Orf6 transgene consisting of the Ad5 Orf6 coding region flanked by the HCMV promoter and pA was cloned into the E1 deletion in an Ad17 shuttle vector (a precursor to the Ad17 ITR cassette). The Ad5 Orf6 transgene was cloned between bp 414 and 3373 in the E1 anti-parallel

orientation. The shuttle vector containing the Ad5 Orf6 transgene was digested to generate a DNA fragment consisting of the transgene flanked by Ad17 sequences (bp 4 to 414 and bp 3373 to 4580) and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pAd24ΔE1, which had been linearized in the E1 region by digestion with *Swa*I, resulted in the generation of pAd24ΔE1Ad5Orf6 by homologous recombination (Figure 13). Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1Ad5Orf6.

In order to determine if pre-adenovirus plasmid pAd24ΔE1Ad5Orf6 could be rescued into virus and propagated in an Ad5/group C E1 complementing cell line, pAd24ΔE1Ad5Orf6 was digested with *Pme* I and transfected into a 6 cm dish of 293 cells using the calcium phosphate co-precipitation technique. *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into 293 cells. Once complete viral cytopathic effect (CPE) was observed at approximately 7-10 days post transfection, the infected cells and media were freeze/thawed three times and the cell debris pelleted. The virus was amplified in two additional passages in 293 cells and then purified from the final infection by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the virus, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hin*dIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pme*1/*Hin*dIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued.

EXAMPLE 9

Insertion of Ad5 Orf 6 into the E4 region of Ad24

To refine the strategy of including Ad5 Orf6 in the genome of an alternative serotype so that propagation could take place in an Ad5/group C complementing cell line two additional strategies were developed. In the first strategy, the entire alternative serotype E4 region (not including the E4 promoter) was deleted and replaced with Ad5 Orf6. In the second strategy, just the alternative serotype Orf6 gene was deleted and replaced with Ad5 Orf6. The configuration of the E4 regions generated by the two strategies is diagramed in Figure 14. For each of these strategies the desired pre-Adenovirus plasmid was generated by bacterial recombination. Cotransformation of BJ 5183 bacteria with pAd24ΔOrf6BstZ17I and the

appropriately constructed Ad24 E4 shuttle plasmid resulted in the generation of the desired Ad24 based pre-Ad plasmid. PAd24ΔOrf6BstZ17I, a derivative of pAd24ΔE1, was constructed so that the E4 region in the Ad24 pre-Ad plasmid could be easily modified using bacterial recombination. PAd24ΔOrf6BstZ17I contains a deletion in the E4 region from Ad24 bp 32373 to bp 33328 with a unique *Bst*Z17I site located at the position of the deletion. The complete sequence of pAd24ΔOrf6BstZ17I consists of Ad17 sequences from bp 4 to 414 and from bp 3373 to 4580, Ad24 bp 4588 to 32372 and from 33329 to 34529, and Ad17 bp 34469 to 35098 (bp numbers refer to the wt sequence for both Ad17 and Ad24).

To construct pAd24ΔE1ΔE4Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1 deletion and a deletion of E4 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed by digesting pAd24ΔE1 with *Pme*I and *Bsr*GI and cloning the restriction fragment representing the E4 region (bp 31559 to bp 35164) into pNEB193, generating pNEBAd24E4. PNEBAd24E4 was then digested with *Acc*I and *Eco*NI to remove the E4 coding sequences and ligated with an oligo designed to contain *Bgl*II and *Xho*I sites (underlined) (5' ACTCGAGATGTATAGATCT (SEQ ID NO: 6); 5' CTAGATCTATACATCTCGAG (SEQ ID NO: 7)), generating pNEBAd24ΔE4. PNEBAd24ΔE4 was then digested with *Bgl*II and *Xho*I and ligated with the Ad5 Orf6 gene, which was PCR amplified, generating pNEBAd24ΔE4Ad5Orf6. The PCR primers used to amplify the Ad5 Orf6 gene (5' GCACAGATCTTTGCTTCAGGAATATG (SEQ ID NO: 8); 5' GAGAACTCGAGGCCTACATGGGGGTAGAG (SEQ ID NO: 9)) were designed to contain *Bgl*II and *Xho*I sites (underlined above) for ligation with the pNEBAd24DE4 fragment. In the final step pNEBAd24ΔE4Ad5Orf6 E4 shuttle plasmid was digested with *Pvu*I and *Pme*I, the restriction fragments were size fractionated by agarose gel electrophoresis and the desired fragment containing Ad5Orf6 flanked by Ad24 sequences was gel purified. Cotransformation of BJ 5183 bacteria with E4 shuttle fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with *Bst*Z17I, resulted in the generation of pAd24ΔE1ΔE4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1ΔE4Ad5Orf6.

To construct pAd24ΔE1ΔOrf6Ad5Orf6 (An Ad24 pre-Ad plasmid containing an E1 deletion and a deletion of E4 Orf6 substituted with Ad5 Orf6), an Ad24 E4 shuttle plasmid was constructed in which the Ad24 Orf6 gene was replaced by Ad5 Orf6. To do this the *Eco*R1 restriction fragment representing bp 32126 to bp 33442 of the Ad24 genome (encompassing the E4 Orf6 coding region), was subcloned into the *Eco*RI site in pNEB193, generating pNEBAd24Orf6. In order to delete the E4 Orf6 gene in pNEBAd24Orf6 and replace it with Ad5 Orf6, pNEBAd24Orf6 was digested with *Sty*I and treated with Klenow to blunt the ends and then

digested with to *Eag*I. The desired pNEBAd24Orf6 fragment was then ligated with a PCR product representing the Ad5 Orf6 gene from Ad5 bp 33193 to bp 24125, generating pNEBAd24ΔOrf6Ad5Orf6. The PCR primers used to generate the Ad5 Orf6 fragment (5'CGAGACGGCCGACGCAGATCTGTTTG (SEQ ID NO: 10); 5'GAAGTCCCGGGCTACATGGGGGTAG (SEQ ID NO: 11)) were designed to contain *Eag*I and *Sma*I sites (underlined above) for ligation with the pNEBAd24Orf6 fragment. In the final step pNEBAd24ΔOrf6Ad5Orf6 was digested with *Eco*RI, the restriction fragments were size fractionated by agarose gel electrophoresis and the desired fragment containing Ad5Orf6 flanked by Ad24 sequences was gel purified. Cotransformation of BJ 5183 bacteria with the *Eco*RI fragment and pAd24ΔOrf6BstZ17I, which had been linearized in the E4 region by digestion with *Bst*Z17I, resulted in the generation of pAd24ΔE1ΔOrf6Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis and one clone was selected as pre-adenovirus plasmid pAd24ΔE1ΔOrf6Ad5Orf6.

EXAMPLE 10

Rescue of pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6, into Virus

In order to determine if pre-adenovirus plasmids pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6, could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmids were each digested with *Pme* I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique; (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring, was observed for both constructs. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect T-225 flasks of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hin*dIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared

with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme*1/*Hind*III prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 11

Comparison of the Growth Kinetics of Ad24 based vectors.

In order to compare the growth kinetic of Ad24ΔE1, Ad24ΔE1Ad5Orf6, Ad24ΔE1ΔE4Ad5Orf6 and Ad24ΔE1ΔOrf6Ad5Orf6 one step growth curves were preformed (Figure 15). PER.C6 cells in 60 mm dishes were infected at 1 vp per cell with either Ad24ΔE1, Ad24ΔE1Ad5Orf6, Ad24ΔE1ΔE4Ad5Orf6 or Ad24ΔE1ΔOrf6Ad5Orf6. Cells and media were then harvested at various times post infection, freeze thawed three times and clarified by centrifugation. The amount of virus present in the samples was determined by quantitative PCR and is illustrated in Figure 15. This study demonstrates that Ad24 vectors that incorporate Ad5 Orf6 have a distinct growth advantage over Ad24ΔE1 in PER.C6 cells. The instant invention can be practiced with recombinant Ad24 vectors absent a heterologous Orf 6 region where the E1-complementing cell line expresses an Ad24 E1 region or, alternatively, E1 and E4 regions of the same serotype (such as Ad5E1/E4-expressing cell lines).

EXAMPLE 12

Insertion of an Expression Cassette into pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6,

In order to introduce a gag or SEAP expression cassette (*see* Figures 6 and 7, respectively) into the E1 region of the Ad24 pre-Adenovirus plasmids described above (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6) bacterial recombination was used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, was cloned into the E1 deletion in Ad17 shuttle plasmid, pABSAd17-3, generating pABSAd17HCMVgagBGHpA. The ITR cassette contains sequences from the right (bp 34469 to 35098) and left (bp 4 to 414 and bp 3373 to 4580) end of the Ad17 genome separated by plasmid sequences containing a bacterial origin of replication and an Ampicillin resistance gene. The ITR cassette contains a deletion of E1 sequences from Ad17 (bp 415 to 3372) with a unique *Swa* I site located in the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the SwaI site in pABSAd17-3. This cloning step resulted in the gag expression cassette being

cloned into the E1 deletion between bp 414 and 3373 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad17 bp 4 to 414 and bp 3373 to 4580 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad24 pre-Ad plasmids (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6,), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the corresponding Ad24 gag-containing pre-Adenovirus plasmids (pAd24ΔE1gagΔE4Ad5Orf6, pAd24ΔE1gagΔOrf6Ad5Orf6) by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad24 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence was cloned into the E1 deletion in Ad17 shuttle plasmid, pABSAd17-3, generating pABSAd17HCMVSEAPBGH. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by EcoRI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pABSAd17-3. The shuttle vector containing the SEAP transgene was digested to generate a DNA fragment consisting of the SEAP expression cassette flanked by Ad17 bp 4 to 414 and bp 3373 to 4580 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and one of the Ad24 pre-Ad plasmids (pAd24ΔE1ΔE4Ad5Orf6, pAd24ΔE1ΔOrf6Ad5Orf6,), linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the corresponding Ad24 SEAP-containing pre-Adenovirus plasmids (pAd24ΔE1SEAPΔE4Ad5Orf6, pAd24ΔE1SEAPΔOrf6Ad5Orf6) by homologous recombination. Potential clones were screened by restriction analysis. All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

EXAMPLE 13

In Vivo Immunogenicity

A. Immunization

Cohorts of 3-6 animals were given intramuscular injections at wk 0 and wk 4 of either of the following constructs: (1) 10^11 vp MRKAd5-HIV1 gag; (2) 10^10 vp MRKAd5-HIV1 gag; (3) 10^11 vp of Ad24ΔE1gagΔOrf6Ad5Orf6; (4) 10^10 vp of

Ad24ΔE1gagΔOrf6Ad5Orf6; or (5) 10^10 vp of Ad24ΔE1gagΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points (typically 4 wk intervals) during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. ELISPOT Assay

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen et al., 2001 *J. Virol.* 75(2):738-749; Casimiro et al., 2002 *J. Virol.* 76:185-94), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Synpep Corp., Dublin, CA). To each well, 50 μL of 2-4 x 10^5 peripheral blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower size cut-off set at 80 femtoliters ("fL"). Either 50 μL of media or the gag peptide pool at 8 μg/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO_2 for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10^6 cell input.

C. Intracellular Cytokine Staining

To 1 ml of 2 x 10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μg/mL. For gag-specific stimulation, 10 μL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO_2, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μL anti-hCD8-PerCP, clone SK1 (Becton Dickinson);

and 20 μL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μL 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both $CD4^+$ and $CD8^+$ populations, and for both mock and gag-peptide reaction tubes of a sample.

D. Anti-p24 ELISA

A modified competitive anti-p24 assay was developed using reagents from the Coulter p24 Antigen Assay kit (Beckman Coulter, Fullerton, CA). Briefly, to a 250-μL serum sample, 20 μL of Lyse Buffer and 15 μL of p24 antigen (9.375 pg) from the Coulter kit were added. After mixing, 200 μL of each sample were added to wells coated with a mouse anti-p24 mAb from the Coulter kit and incubated for 1.5 hr at 37°C. The wells were then washed and 200 μL of Biotin Reagent (polyclonal anti-p24-biotin) from the Coulter kit was added to each well. After a 1 hr, 37°C incubation, detection was achieved using strepavidin-conjugated horseradish peroxidase and TMB substrate as described in the Coulter Kit. OD_{450nm} values were recorded. A 7-point standard curve was generated using a serial 2-fold dilution of serum from an HIV-seropositive individual. The lower cut-off for the assay is arbitrarily set at 10 milli Merck units/mL (mMU/mL) defined by a dilution of the seropositive human serum. This cutoff falls at approximately 65% of the maximum bound control signal which corresponds to that obtained with the diluent control only and with no positive analyte.

E. Results

PBMCs collected at regular 4-wk intervals were analyzed in an ELISPOT assay (Figure 17). Both Ad24ΔE1gagΔOrf6Ad5Orf6 and Ad24ΔE1gagΔE4Ad5Orf6 were able to induce significant levels of gag-specific T cells in non-human primates. At 10^11 vp dose level, the Ad24-induced responses were within 2-3-fold of those of MRKAd5-HIV1 gag. Both Ad24 vectors were also able to induce detectable levels of gag-specific T cells at 10^10 vp but were lower than those observed using MRKad5gag at the same dose.

PBMCs collected at wk 12 from the vaccinees were analyzed for intracellular IFN-γ staining after the priming immunizations. The assay results provided information on the relative amounts of $CD4^+$ and $CD8^+$ gag-specific T cells in the peripheral blood (Figure 18). The

results indicated that the prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific $CD4^+$ and $CD8^+$ T cells.

F. Humoral Immune Responses

The Ad24-based vaccine vector was able to generate detectable levels of circulating anti-gag antibodies at the reasonably high dose level (Figure 19). No detectable titers were observed at equal to or lower than 10^10 vp, suggesting the existence of a dose-dependent response.

EXAMPLE 14

In Vivo Transgene Expression

A. Immunization

Cohorts of 5 C3H/HeN mice were given single intramuscular injections of one of the following vectors: (1) 10^10 vp Ad24ΔE1SEAPΔE4Ad5Orf6; (2) 10^10 vp Ad24ΔE1SEAPΔOrf6Ad5Orf6; (3) 10^10 vp MRKAd5SEAP; and (4) 10^9 vp MRKAd5SEAP. Female mice were between 4-10 weeks old. The total dose of each vaccine was suspended in 0.1 mL of buffer. The vectors were given to both quadriceps of each of the animals with a volume of 50 uL per quad and using 0.3-mL 28G1/2 insulin syringes (Becton-Dickinson, Franklin Lakes, NJ). For the primates, the total dose of each vaccine was suspended in 1 mL of buffer. The monkeys were anesthetized (ketamine/xylazine mixture) and the vaccines were delivered i.m. in 0.5-mL aliquots into two muscle sites using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Serum samples were collected at defined intervals and stored frozen until the assay date. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

Serum samples were analyzed for circulating SEAP levels using TROPIX phospha-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 uL aliquots of each serum were mixed with 45 uL of kit-supplied dilution buffer in a 96-well white DYNEX plate. Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the wells for 30 minutes at 65 °C.

Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorder using DYNEX luminometer. RLU readings are converted to ng/mL SEAP using a log-log regression analyses.

C. Rodent Results

Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 20. Results indicate that (1) both Ad24 constructs are all capable of expressing the SEAP transgene in vivo to comparable levels; and that (2) the level of expression achieved using the Ad24 vectors are comparable to that of Ad5 at 10-fold lower dose. The levels of SEAP in the serum dropped dramatically after day 2 and were at background levels by day 12.

D. Primate Results

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the following vectors: (1) 10^11 vp MRKAd5-SEAP; (2) 10^9 vp MRKAd5-SEAP; (3) 10^11 vp Ad24ΔE1SEAPΔOrf6Ad5Orf6; or (4) 10^11 vp Ad24ΔE1SEAPΔE4Ad5Orf6. Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 21.

Results indicate that the peak levels of SEAP product produced by adenovirus serotype 24 were lower than but were within 3-fold of that of MRKAd5 at the same high dose level of 10^11 vp (Figure 21). The levels observed with adenovirus serotype 24 are generally 50-fold higher than those observed using 10^9 vp of MRKAd5. The levels of SEAP in the serum dropped dramatically after day 10 and were close to background as early as day 15. These observations strongly indicate that adenovirus serotype 24 is very efficient in expressing a transgene following intramuscular administration in a primate.

EXAMPLE 15

Construction of pMRKAd24ΔE1ΔE4Ad5Orf6

To construct pMRKAd24ΔE1ΔE4Ad5Orf6 (An Ad24 pre-Ad plasmid, composed entirely of Ad24 sequence and containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), an Ad24 ITR cassette was constructed containing sequences from the right (bp 31978 to 32264 and bp 34713 to 35164) and left (bp 4 to 450 and bp 3364 to 3799) end of the Ad24 genome separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd24-4. Next the Ad5 Orf6 open reading frame (Ad5 bp 31192 to bp 34078) was generated by PCR and cloned between Ad24 bp 32264 and 34713 generating

pNEBAd24E-Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad24 bp 451 to 3363 with a unique Swa I restriction site located in the deletion and an E4 deletion from Ad24 bp 32265 to 34712 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5 Orf6 expression is driven by the Ad24 E4 promoter. The Ad24 sequences (bp 31978 to 32264 and bp 3464 to 3799) in the ITR cassette provide regions of homology with the purified Ad24 viral DNA in which bacterial recombination can occur following cotransformation into BJ 5183 bacteria (Figure 22). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion will release the recombinant Ad24 genome from plasmid sequences. Potential clones will be screened by restriction analysis and one clone was selected as pMRKAd24ΔE1ΔE4Ad5Orf6. Pre-Adenovirus plasmid pMRKAd24ΔE1ΔE4Ad5Orf6 should contain Ad24 sequences from bp 4 to 450; bp 3364 to bp 32264 and bp 34713 to bp 35164 with Ad5Orf6 cloned between bp 32264 and bp 34713. The bp numbering in the above description refers to the wt sequence for both Ad24 and Ad5.

EXAMPLE 16

<u>Insertion of HIV-1 gag and SEAP transgenes into pAd24ΔE1ΔE4Ad5Orf6</u>

In order to introduce a gag or SEAP expression cassettes into the E1 region of pMRKAd24ΔE1ΔE4Ad5Orf6, bacterial recombination will be used. An HIV-1 gag expression cassette will consist of the following: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, in the E1 deletion of an Ad24 shuttle plasmid, pNEBAd24-2 (a precursor to the Ad24 ITR cassette described above), generating pNEBAd24CMVgagBGHpA. PNEBAd24-2 contains Ad24 sequences from the left end of the genome (bp 4 to 450 and bp 3364 to 3799) that define the E1 deletion. The gag expression cassette will be obtained from a previously constructed plasmid and cloned into the E1 deletion between bp 450 and 3364 in the E1 parallel orientation. The shuttle vector containing the gag transgene will be digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad24 bp 4 to 450 and bp 3364 to 3799 and the fragment will be purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pMRKAd24ΔE1ΔE4Ad5Orf6 which was linearized in the E1 region by digestion with SwaI, should result in the generation of Ad24 gag-

containing pre-Adenovirus plasmids pMRKAd24ΔE1gagΔE4Ad5Orf6 by homologous recombination. Potential clones will be screened by restriction analysis.

A similar strategy will be used to generate Ad24 pre-Ad plasmids containing a SEAP expression cassette. In this case, a SEAP expression cassette will consist of: 1) the immediate early gene promoter from the human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence cloned into the E1 deletion of an Ad24 shuttle plasmid, pNEBAd24-2, generating pNEBAd24CMVSEAPBGHpA. The transgene will then be recombined into pMRKAd24ΔE1ΔE4Ad5Orf6 as described above for the gag transgene.

EXAMPLE 17

In Vivo *Immunogenicity*

A. Immunization

Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. T Cell Responses

Ad24 Vaccine Vector as a Heterologous Booster: Cohort of 4 rhesus macaques was immunized initially with 3 doses (wk 0, 4, 26) of either 10^7 or 10^9 vp of MRKAd5-gag (*see*, PCT/US01/28861, published March 21, 2002) or MRKAd6-gag. At wk 56, the animals received a booster vaccine of 10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6. A separate cohort of naïve animals received a single dose of the booster vaccine. The results of the IFN-γ ELISPOT analyses of PBMC collected during the course of the studies are shown in Figure 23. It is apparent that the Ad24 HIV vectors can be utilized to amplify the existing pools of HIV-specific T cells. The increases in the levels of gag-specific T cells from the pre-boost levels to those measured at 4 wks post boost were consistently larger than the levels induced by the same booster vaccine in naïve animals. PBMCs from the vaccinees of the heterologous MRKAd5/MRKAd6-Ad24 boost

regimen were analyzed for intracellular IFN-γ staining after the priming immunizations (wk 60). The assay results provided information on the relative amounts of $CD4^+$ and $CD8^+$ gag-specific T cells in the peripheral blood (Figure 24). The results indicated that heterologous prime-boost immunization approach was able to elicit in rhesus macaques both HIV-specific CD4+ and CD8+ T cells.

Ad24 Vaccine Vector as a Heterologous Primer: In a separate study, a cohort of 3 rhesus macaques was immunized initially with 2 doses (wk 0, 4) of 10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6 and boosted at wk 24 with 10^7 vp of MRKAd5-gag. The low dose of MRKAd5-gag is selected to mimic the effect of pre-existing neutralizing immunity to the vector in a subject. A separate cohort of naïve animals was given a single dose of 10^7 vp MRKAd5-gag. The results of the IFN-γ ELISPOT analyses of PBMC collected during the course of the studies are shown in Figure 25.

The Ad24-based vaccine was able to prime effectively for HIV-specific T cell responses in macaques. Boosting with a low dose MRKAd5-gag resulted in a significant increase in the levels of gag-specific T cells. The increases in 2 out of 3 animals exceed the levels typically observed after treatment of naïve animals with the same low dose of MRKAd5-gag.

EXAMPLE 18

Construction of pAd34ΔE1ΔE4Ad5Orf6

To generate an E1- Ad34 based vector that can propagate in existing group C/Ad5 E1 complementing cell lines (293, PER.C6), Ad5 Orf6 was inserted in place of the native E4 region. Since at the time, the complete sequence of Ad34 (*see* Figures 28A-1 to 28A-9; subject of copending application serial no. 60/458,825, filed March 28, 2003) was unknown, advantage was taken of the sequence homology between Ad34 and Ad35 in order to construct the Ad34 pre-Adenovirus plasmid. Cotransformation of BJ 5183 bacteria with purified wild-type Ad34 viral DNA and the appropriately constructed Ad35 ITR cassette resulted in the circularization of the viral genome by homologous recombination. The construction of the pre-Ad plasmid based on Ad34, is outlined below:

To construct pAd34ΔE1ΔE4Ad5Orf6 (An Ad34 pre-Ad plasmid containing an E1 deletion and an E4 deletion substituted with Ad5 Orf6), we utilized an Ad35 ITR cassette. We anticipated that sequence homology between Ad34 and Ad35 would allow homologous recombination to occur. The Ad35 ITR cassette was constructed containing sequences from the right (bp 31599 to 31913 and bp 34419 to 34793) and left (bp 4 to 456 and bp 3403 to 3886) end of the Ad35 genome (*see* Figures 2A-1 to 2A-10) separated by plasmid sequences containing a

bacterial origin of replication and an ampicillin resistance gene. The four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd35-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad35 bp 31913 and 34419 generating pNEBAd35-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad35 bp 457 to 3402 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad35 bp 31914 to 34418 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5Orf6 expression is driven by the Ad35 E4 promoter. The Ad35 sequences (bp 31599 to 31913 and bp 3403 to 3886) in the ITR cassette provided regions of homology with the purified Ad34 viral DNA in which bacterial recombination could occur following cotransformation into BJ 5183 bacteria (Figure 26). The ITR cassette was also designed to contain unique restriction enzyme sites (*Pme*I) located at the end of the viral ITR's so that digestion would release the recombinant Ad34 genome from the plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pAd34ΔE1ΔE4Ad5Orf6.

EXAMPLE 19

Rescue of pAd34ΔE1ΔE4Ad5Orf6 into Virus

In order to determine if pre-adenovirus plasmid pAd34ΔE1ΔE4Ad5Orf6, could be rescued into virus and propagated in a group C E1 complementing cell line, the plasmid was digested with *Pme* I and transfected into T-25 flasks of PER.C6 cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc). *Pme*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after cell entry. Viral cytopathic effect (CPE), indicating that virus replication and amplification was occurring was observed following transfection. When CPE was complete, approximately 7-10 days post transfection, the infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Approximately 1 ml of the cell lysate was used to infect a T-225 flask of PER.C6 cells at 80-90% confluence. Once CPE was reached, infected cells and media were harvested, freeze/thawed three times and the cell debris pelleted by centrifugation. Clarified cell lysates were then used to infect 2-layer NUNC cell factories of PER.C6 cells. Following complete CPE, the virus was purified by ultracentrifugation on CsCl density gradients. In order to verify the genetic structure of the rescued viruses, viral DNA was extracted using pronase treatment

followed by phenol chloroform extraction and ethanol precipitation. Viral DNA was then digested with *Hin*dIII and treated with Klenow fragment to end-label the restriction fragments with P33-dATP. The end-labeled restriction fragments were then size-fractionated by gel electrophoresis and visualized by autoradiography. The digestion products were compared with the digestion products of the corresponding pre-Adenovirus plasmid (that had been digested with *Pme*1/*Hind*III prior to labeling) from which they were derived. The expected sizes were observed, indicating that the viruses had been successfully rescued.

EXAMPLE 20

Insertion of an Expression Cassette into pAd34ΔE1ΔE4Ad5Orf6

In order to introduce a gag or SEAP expression cassette (*see* Figures 6 and 7, respectively) into the E1 region of pAd34ΔE1ΔE4Ad5Orf6, bacterial recombination was again used. A gag expression cassette consisting of the following: 1) the immediate early gene promoter from human cytomegalovirus, 2) the coding sequence of the human immunodeficiency virus type 1 (HIV-1) gag (strain CAM-1; 1526 bp) gene, and 3) the bovine growth hormone polyadenylation signal sequence, was cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2 (a precursor to the Ad35 ITR cassettes described above), generating pNEBAd35CMVgagBGHpA. pNEBAd35-2 contains Ad35 sequences from the left end of the genome (bp 4 to 456 and bp 3403 to 3886) with a unique *Swa*I site between bp 456 and 3403 at the position of the deletion. The gag expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pNEBAd35-2. This cloning step resulted in the gag expression cassette being inserted into the E1 deletion between bp 456 and 3403 in the E1 parallel orientation. The shuttle vector containing the gag transgene was digested to generate a DNA fragment consisting of the gag expression cassette flanked by Ad35 bp 4 to 456 and bp 3403 to 3886 and the fragment was purified after electrophoresis on an agarose gel. Cotransformation of BJ 5183 bacteria with the shuttle vector fragment and pAd34ΔE1ΔE4Ad5Orf6, linearized in the E1 region by digestion with *Swa* I, resulted in the generation of the Ad34 gag-containing pre-Adenovirus plasmid pAd34ΔE1gagΔE4Ad5Orf6 by homologous recombination. Potential clones were screened by restriction analysis.

A similar strategy was used to generate Ad34 pre-Ad plasmids containing a SEAP expression cassette. In this case a SEAP expression cassette consisting of: 1) the immediate early gene promoter from human cytomegalovirus, 2) the coding sequence of the human placental SEAP gene, and 3) the bovine growth hormone polyadenylation signal sequence was

cloned into the E1 deletion in Ad35 shuttle plasmid, pNEBAd35-2, generating pNEBAd35CMVSEAPBGHpA. The SEAP expression cassette was obtained from a previously constructed shuttle plasmid by *Eco*RI digestion. Following the digestion the desired fragment was gel purified, treated with Klenow to obtain blunt ends and cloned into the *Swa*I site in pNEBAd35-2. The transgene was then recombined into the pAd34ΔE1ΔE4Ad5Orf6, generating pAd34ΔE1SEAPΔE4Ad5Orf6 as described above for the gag transgene.

All pre-Ad plasmids were rescued into virus and expanded to prepare CsCl purified stocks as described above.

EXAMPLE 21

Construction of pMRKAd34ΔE1ΔE4Ad5Orf6

To construct an Ad34 pre-Ad plasmid that was composed entirely of Ad34 sequences, an Ad34 ITR cassette was generated. The Ad34 ITR cassette was constructed containing sequences from the right (bp 31584 to 31895 and bp 34409 to 34772) and left (bp 4 to 456 and bp 3402 to 3885) end of the Ad34 genome (*see* Figures 28A-1 to 28A-9) separated by plasmid sequences containing a bacterial origin of replication and an ampicillin resistance gene. These four segments were generated by PCR and cloned sequentially into pNEB193, generating pNEBAd34-4. Next the Ad5 Orf6 open reading frame was generated by PCR and cloned between Ad34 bp 31895 and 34409 generating pNEBAd34-4Ad5Orf6 (the ITR cassette). PNEB193 is a commonly used commercially available cloning plasmid (New England Biolabs cat# N3051S) containing a bacterial origin of replication, ampicillin resistance gene and a multiple cloning site into which the PCR products were introduced. The ITR cassette contains a deletion of E1 sequences from Ad34 bp 457 to 3401 with a unique *Swa* I restriction site located in the deletion and an E4 deletion from Ad34 bp 31896 to 34408 into which Ad5 Orf6 was introduced in an E4 parallel orientation. In this construct Ad5Orf6 expression is driven by the Ad34 E4 promoter. The Ad34 sequences (bp 31584 to 31895 and bp 3402 to 3885) in the ITR cassette provided regions of homology with the purified Ad34 viral DNA in which bacterial recombination could occur following cotransformation into BJ 5183 bacteria (Figure 27). The ITR cassette was also designed to contain unique restriction enzyme sites (PmeI) located at the end of the viral ITR's so that digestion would release the recombinant Ad34 genome from the plasmid sequences. Potential clones were screened by restriction analysis and one clone was selected as pMRKAd34ΔE1ΔE4Ad5Orf6.

EXAMPLE 22

In Vivo Studies

A. Immunization

Cohorts of 3 rhesus macaques were given single intramuscular injections of one of the two vectors: (1) 10^11 vp MRKAd5-SEAP (in MRKAd vector backbone disclosed in PCT/US01/28861, published March 21, 2002); and (2) 10^11 vp Ad34ΔE1SEAPΔE4Ad5Orf6. Rhesus macaques were between 3-10 kg in weight. In all cases, the total dose of each vaccine was suspended in 1 mL of buffer. The macaques were anesthetized (ketamine/xylazine) and the vaccines were delivered i.m. in 0.5-mL aliquots into both deltoid muscles using tuberculin syringes (Becton-Dickinson, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were prepared from blood samples collected at several time points during the immunization regimen. All animal care and treatment were in accordance with standards approved by the Institutional Animal Care and Use Committee according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council.

B. SEAP Assay

Serum samples were analyzed for circulating human secreted alkaline phosphatase (SEAP) levels using TROPIX phospha-light chemiluminescent kit (Applied Biosystems Inc). Duplicate 5 μL aliquots of each serum were mixed with 45 μL of kit-supplied dilution buffer in a 96-well white DYNEX plate. Serially diluted solutions of a human placental alkaline phosphatase (Catalog no. M5905, Sigma, St. Louis, MO) in 10% naïve monkey serum served to provide the standard curve. Endogenous SEAP activity in the samples was inactivated by heating the well for 30 minutes at 65 °C. Enzymatic SEAP activities in the samples were determined following the procedures described in the kit. Chemiluminescence readings (in relative light units) were recorded using DYNEX luminometer. RLU readings were converted to ng/mL SEAP using a log-log regression analyses.

C. ELISPOT Assay

The IFN-γ ELISPOT assays for rhesus macaques were conducted following a previously described protocol (Allen *et al.*, 2001 *J. Virol.* 75(2):738-749), with some modifications. For antigen-specific stimulation, a peptide pool was prepared from 20-aa peptides that encompass the entire HIV-1 gag sequence with 10-aa overlaps (Sympep Corp., Dublin, CA). To each well, 50 μL of 2-4 x 10^5 peripheral blood mononuclear cells (PBMCs) were added; the cells were counted using Beckman Coulter Z2 particle analyzer with a lower

size cut-off set at 80 femtoliters ("fL"). Either 50 μL of media or the gag peptide pool at 8 μg/mL concentration per peptide was added to the PBMC. The samples were incubated at 37°C, 5% CO_2 for 20-24 hrs. Spots were developed accordingly and the plates were processed using custom-built imager and automatic counting subroutine based on the ImagePro platform (Silver Spring, MD); the counts were normalized to 10^6 cell input.

D. Intracellular Cytokine Staining (ICS)

To 1 ml of 2 x 10^6 PBMC/mL in complete RPMI media (in 17x100mm round bottom polypropylene tubes (Sarstedt, Newton, NC)), anti-hCD28 (clone L293, Becton-Dickinson) and anti-hCD49d (clone L25, Becton-Dickinson) monoclonal antibodies were added to a final concentration of 1 μg/mL. For gag-specific stimulation, 10 μL of the peptide pool (at 0.4 mg/mL per peptide) were added. The tubes were incubated at 37 °C for 1 hr., after which 20 μL of 5 mg/mL of brefeldin A (Sigma) were added. The cells were incubated for 16 hr at 37 °C, 5% CO_2, 90% humidity. 4 mL cold PBS/2%FBS were added to each tube and the cells were pelleted for 10 min at 1200 rpm. The cells were re-suspended in PBS/2%FBS and stained (30 min, 4 °C) for surface markers using several fluorescent-tagged mAbs: 20 μL per tube anti-hCD3-APC, clone FN-18 (Biosource); 20 μL anti-hCD8-PerCP, clone SK1 (Becton Dickinson); and 20 μL anti-hCD4-PE, clone SK3 (Becton Dickinson). Sample handling from this stage was conducted in the dark. The cells were washed and incubated in 750 μL 1xFACS Perm buffer (Becton Dickinson) for 10 min at room temperature. The cells were pelleted and re-suspended in PBS/2%FBS and 0.1 μg of FITC-anti-hIFN-γ, clone MD-1 (Biosource) was added. After 30 min incubation, the cells were washed and re-suspended in PBS. Samples were analyzed using all four color channels of the Becton Dickinson FACSCalibur instrument. To analyze the data, the low side- and forward-scatter lymphocyte population was initially gated; a common fluorescence cut-off for cytokine-positive events was used for both $CD4^+$ and $CD8^+$ populations, and for both mock and gag-peptide reaction tubes of a sample.

E. Results

Expression: Serum samples prior to and after the injection were analyzed for circulating SEAP activities and the results are shown in Figure 29. Results indicate that the peak levels of SEAP protein produced by the alternative adenovirus serotype were lower than but were within 3-fold of that of MRKAd5 at the same high dose level of 10^11 vp (Figure 29). The levels of SEAP in the serum dropped dramatically after day 10 and were close to background as early as day 15. These observations strongly indicate that the Ad34-based vector is efficient in expressing a transgene following intramuscular administration in a primate.

Immunogenicity: Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 30; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool or the mock (no peptide) control.

Immunization with gag-expressing Ad34 vector induced detectable levels of circulating gag-specific T cells immediately after a single dose of the vector. The responses improved following a second dose given at wk 4. Overall, the responses to the Ad34-based vector were slightly lower than those induced by the same dose of MRKAd5-gag. The results strongly indicate the Ad34-based vector can prime effectively for HIV-specific T cell responses.

IFN-γ ICS analyses of the PBMC from the Ad34-immunized animals revealed that the vector can induce detectable levels of both $CD4^+$ and $CD8^+$ HIV-specific T cells (Figure 31).

EXAMPLE 23

Heterologous Immunization

Cohorts of 3 monkeys were immunized (at wks 0, 4) with 10^11 vp Ad34ΔE1gagΔE4Ad5Orf6 followed by a booster at week 24 with 10^10 vp Ad35ΔE1gagΔE4Ad5Orf6. Vaccine-induced T cell responses against HIV-1 gag were quantified using IFN-gamma ELISPOT assay against a pool of 20-aa peptides that encompassed the entire protein sequence. The results are shown in Figure 32; they are expressed as the number of spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMCs) that responded to the peptide pool or the mock (no peptide) control.

Immunization with gag-expressing Ad34 vector induced detectable levels of circulating gag-specific T cells that decreased to between 94-139 SFC/10^6 PBMC at the time of the boost. Heterologous immunization with an Ad35-based HIV vector resulted in as much as a 3-fold increase in T cell responses.

IFN-γ ICS analyses of the PBMCs from the Ad34 primed/Ad35 boosted animals at week 28 revealed that the vector can induce detectable levels of both $CD4^+$ and $CD8^+$ HIV-specific T cells (Figure 33).

WHAT IS CLAIMED IS:

1. A means for propagating replication-defective adenovirus in an adenoviral E1-complementing cell line expressing E1 gene product(s) which are non-native to the adenovirus, which comprises:

(a) inserting all or a portion of a heterologous adenoviral E4 region comprising nucleic acid sequence encoding open reading frame 6 (ORF6) into a replication-defective adenovirus; wherein the E4 region or portion thereof inserted into the adenovirus is native to a virus of the same adenovirus serotype as the E1 gene product(s) expressed by the complementing cell line;

(b) introducing the replication-defective adenovirus into the adenoviral E1-complementing cell line;

(c) allowing the replication-defective adenovirus to propagate in the adenoviral E1-complementing cell line; and

(d) rescuing the propagated adenovirus.

2. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof comprises the complete adenoviral E4-encoding region.

3. A means in accordance with claim 2 wherein the heterologous adenoviral E4 region or portion thereof comprises the complete adenoviral E4-encoding region and native E4 promoter.

4. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is inserted into the replication-defective virus in place of nucleic acid sequence encoding open reading frame 6 (ORF6).

5. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is inserted into the replication-defective virus in place of nucleic acid sequence encoding the complete adenoviral E4-encoding region.

6. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is derived from a subgroup C adenovirus.

7. A means in accordance with claim 1 wherein the subgroup C adenovirus is adenovirus of serotype 5.

8. A means in accordance with claim 7 wherein the replication-defective adenovirus is an adenovirus of subgroup B.

9. A means in accordance with claim 7 wherein the replication-defective adenovirus is an adenovirus of serotype 35.

10. A means in accordance with claim 1 wherein the heterologous adenoviral E4 region or portion thereof is operatively linked to a heterologous promoter.

11. A means in accordance with claim 1 wherein the adenoviral E1-complementing cell line is a PER.C6® cell line.

12. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6).

13. A replication-defective adenovirus in accordance with claim 12 wherein the adenovirus comprises a heterologous gene of interest.

14. A replication-defective adenovirus in accordance with claim 13 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

15. A replication-defective adenovirus in accordance with claim 14 wherein the HIV-1 antigen is selected from the group consisting of HIV-1 gag, pol, nef and env.

16. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6) and a gene encoding HIV-1 gag.

17. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a heterologous adenoviral open reading frame 6 (ORF6) in place of a native E4 region or portion thereof comprising ORF6.

18. A replication-defective adenovirus comprising all or a portion of a heterologous E4 region comprising a complete heterologous E4 region in place of a complete native E4 region.

19. A replication-defective adenovirus comprising a heterologous E4 region or portion thereof comprising a complete heterologous E4 region including E4 promoter in place of a complete native E4 region.

20. Adenovirus propagated in accordance with the means of claim 1.

21. A means in accordance with claim 1 wherein the replication-defective adenovirus comprises a heterologous gene of interest.

22. A means in accordance with claim 21 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

23. A means in accordance with claim 22 wherein the HIV-1 antigen is selected from the group consisting of: HIV-1 gag, pol, nef and env.

24. A replication-defective adenovirus of serotype 35 comprising all or a portion of an adenovirus serotype 5 E4 region comprising open reading frame 6 (ORF6) and a heterologous gene of interest.

25. A replication-defective adenovirus in accordance with claim 24 wherein the heterologous gene of interest is a gene encoding an HIV-1 antigen.

26. A replication-defective adenovirus in accordance with claim 25 wherein the HIV-1 antigen is selected from the group consisting of: HIV-1 gag, pol, nef and env.

27. A replication-defective adenovirus of serotype 35 comprising all or a portion of an adenovirus serotype 5 E4 region comprising open reading frame 6 (ORF6) and a gene encoding HIV-1 gag.

28. A recombinant adenoviral vector of serotype 24 which comprises an E4 gene or a segment of an E4 gene comprising open reading frame 6 ("ORF6") of an alternative serotype.

29. A population of cells comprising the recombinant adenoviral vector of claim 28.

30. A method for producing recombinant, replication-defective adenovirus particles comprising:

(a) introducing a recombinant adenoviral vector of claim 28 into a population of cells expressing adenovirus E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

31. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 30.

32. A composition comprising purified recombinant adenovirus particles in accordance with claim 31.

33. A composition in accordance with claim 32 which comprises a physiologically acceptable carrier.

34. A recombinant adenoviral vector in accordance with claim 28 which is at least partially deleted in E1 and devoid of E1 activity and comprises a heterologous nucleic acid.

35. A composition comprising purified recombinant adenoviral particles in accordance with claim 31 which are at least partially deleted in E1 and devoid of E1 activity and comprise a heterologous nucleic acid.

36. A method for effecting the delivery and expression of heterologous nucleic acid comprising administering the composition of claim 35 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

37. A method in accordance with claim 36 wherein the composition is preceded or followed by administration of heterologous nucleic acid with an adenovirus of a different serotype.

38. A composition in accordance with claim 35 wherein the heterologous nucleic acid encodes an HIV antigen.

39. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 38.

40. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

41. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

42. A composition in accordance with claim 39 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

43. A recombinant adenoviral vector of serotype 24 which is at least partially deleted in E1 and devoid of E1 activity; wherein said vector comprises an E4 gene or a segment of an E4 gene from adenovirus serotype 5 comprising open reading frame 6 ("ORF6"), and a heterologous nucleic acid.

44. A population of cells comprising the recombinant adenoviral vector of claim 43.

45. A method for producing recombinant, replication-defective adenovirus particles comprising:

(a) introducing a recombinant adenoviral vector of claim 43 into a population of cells expressing adenovirus serotype 5 E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

46. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 45.

47. A composition comprising purified recombinant adenovirus particles in accordance with claim 46.

48. A composition in accordance with claim 47 which comprises a physiologically acceptable carrier.

49. A method for effecting the delivery and expression of the heterologous nucleic acid comprising administering the composition of claim 48 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

50. A method in accordance with claim 49 above wherein the composition is preceded or followed by administration of the heterologous nucleic acid with an adenovirus of a different serotype.

51. A composition in accordance with claim 48 wherein the heterologous nucleic acid encodes an HIV antigen.

52. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 51.

53. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

54. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

55. A composition in accordance with claim 51 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

56 A recombinant adenoviral vector of serotype 34 which comprises an E4 gene or a segment of an E4 gene comprising open reading frame 6 ("ORF6") of an alternative serotype.

57. A population of cells comprising the recombinant adenoviral vector of claim 56.

58. A method for producing recombinant, replication-defective adenovirus particles comprising:

(a) introducing a recombinant adenoviral vector of claim 56 into a population of cells expressing adenovirus E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

59. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 58.

60. A composition comprising purified recombinant adenovirus particles in accordance with claim 59.

61. A composition in accordance with claim 60 which comprises a physiologically acceptable carrier.

62. A recombinant adenoviral vector in accordance with claim 56 which is at least partially deleted in E1 and devoid of E1 activity and comprises a heterologous nucleic acid.

63. A composition comprising purified recombinant adenoviral particles in accordance with claim 59 which are at least partially deleted in E1 and devoid of E1 activity and comprise a heterologous nucleic acid.

64. A method for effecting the delivery and expression of heterologous nucleic acid comprising administering the composition of claim 63 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

65. A method in accordance with claim 64 wherein the composition is preceded or followed by administration of heterologous nucleic acid with an adenovirus of a different serotype.

66. A composition in accordance with claim 63 wherein the heterologous nucleic acid encodes an HIV antigen.

67. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 66.

68. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

69. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

70. A composition in accordance with claim 67 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

71. A recombinant adenoviral vector of serotype 34 which is at least partially deleted in E1 and devoid of E1 activity; wherein said vector comprises an E4 gene or a segment of an E4 gene from adenovirus serotype 5 comprising open reading frame 6 ("ORF6"), and a heterologous nucleic acid.

72. A population of cells comprising the recombinant adenoviral vector of claim 71.

73. A method for producing recombinant, replication-defective adenovirus particles comprising:

(a) introducing a recombinant adenoviral vector of claim 71 into a population of cells expressing adenovirus serotype 5 E1; and

(b) harvesting the resultant recombinant, replication-defective adenovirus.

74. Purified recombinant, replication-defective adenovirus particles harvested in accordance with the method of claim 73.

75. A composition comprising purified recombinant adenovirus particles in accordance with claim 74.

76. A composition in accordance with claim 75 which comprises a physiologically acceptable carrier.

77. A method for effecting the delivery and expression of the heterologous nucleic acid comprising administering the composition of claim 76 prior or subsequent to administration of the heterologous nucleic acid with the same or different vector.

78. A method in accordance with claim 77 above wherein the composition is preceded or followed by administration of the heterologous nucleic acid with an adenovirus of a different serotype.

79. A composition in accordance with claim 76 wherein the heterologous nucleic acid encodes an HIV antigen.

80. A method for generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a composition of claim 79.

81. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 gag or immunologically relevant modification thereof.

82. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 nef or immunologically relevant modification thereof.

83. A composition in accordance with claim 79 wherein the HIV antigen is HIV-1 pol or immunologically relevant modification thereof.

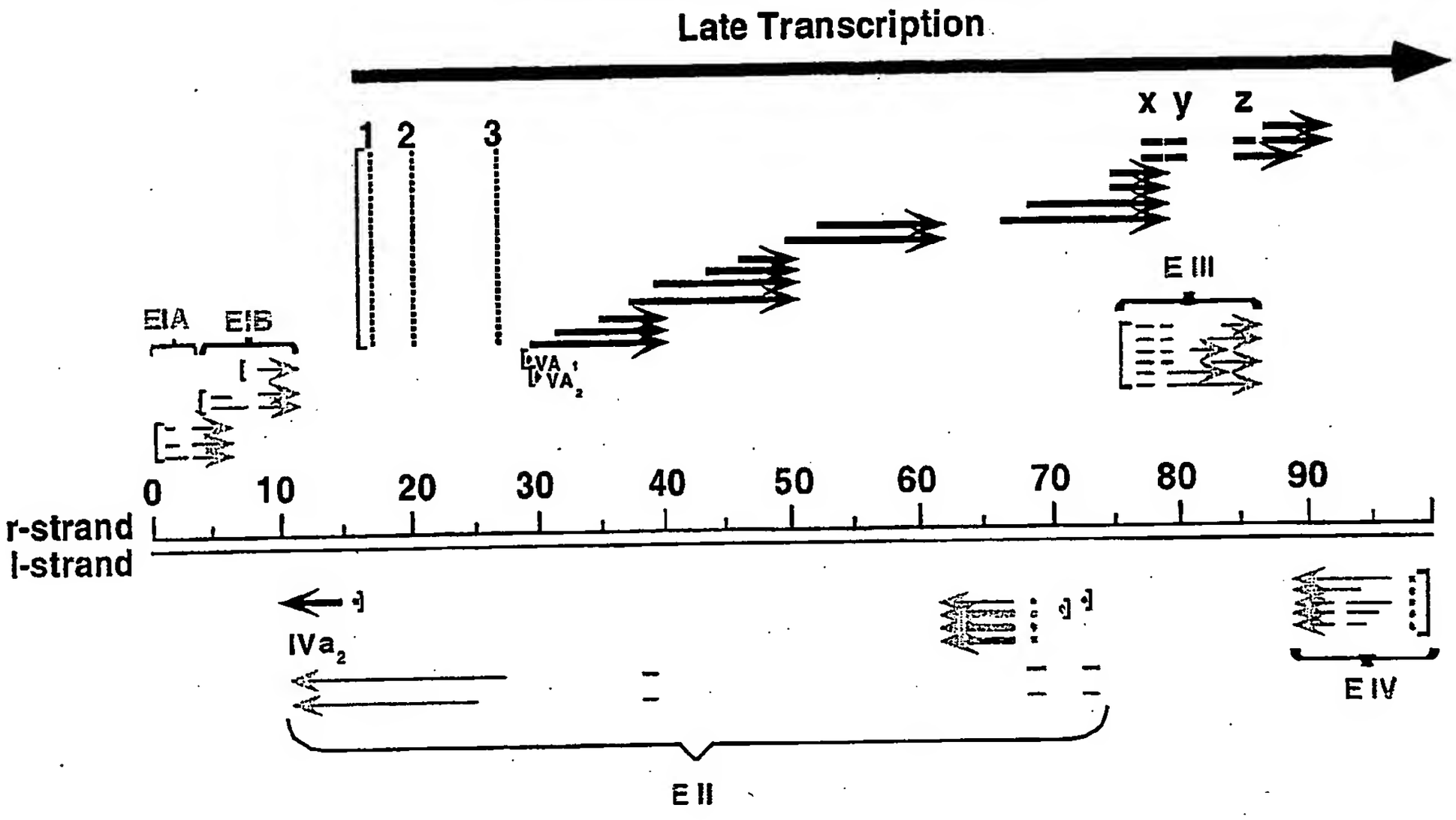

Late Transcription
1
2
3
x
y
z
E1A
E1B
pVA1
pVA2
E III
0
10
20
30
40
50
60
70
80
90
r-strand
l-strand
IVa2
E II
E IV

FIG. 1

```
   1 catcatcaat aatatacctt atagatggaa tggtgccaat atgtaaatga ggtgatttta
  61 aaaagtgtgg gccgtgtggt gattggctgt ggggttaacg gttaaaaggg gcggcgcggc
 121 cgtgggaaaa tgacgtttta tgggggtgga gtttttttgc aagttgtcgc gggaaatgtt
 181 acgcataaaa aggcttcttt tctcacggaa ctacttagtt ttcccacggt atttaacagg
 241 aaatgaggta gttttgaccg gatgcaagtg aaaattgctg attttcgcgc gaaaactgaa
 301 tgaggaagtg tttttctgaa taatgtggta tttatggcag ggtggagtat ttgttcaggg
 361 ccaggtagac tttgacccat tacgtggagg tttcgattac cgtgtttttt acctgaattt
 421 ccgcgtaccg tgtcaaagtc ttctgttttt acgtaggtgt cagctgatcg ctagggtatt
 481 tatacctcag ggtttgtgtc aagaggccac tcttgagtgc cagcgagaag agttttctcc
 541 tctgcgccgg cagtttaata ataaaaaaat gagagatttg cgatttctgc ctcaggaaat
 601 aatctctgct gagactggaa atgaaatatt ggagcttgtg gtgcacgccc tgatgggaga
 661 cgatccggag ccacctgtgc agctttttga gcctcctacg cttcaggaac tgtatgattt
 721 agaggtagag ggatcggagg attctaatga ggaagctgtg aatggctttt ttaccgattc
 781 tatgctttta gctgctaatg aaggattaga attagatccg cctttggaca ctttcaatac
 841 tccaggggtg attgtggaaa gcggtacagg tgtaagaaaa ttacctgatt tgagttccgt
 901 ggactgtgat ttgcactgct atgaagacgg gtttcctccg agtgatgagg aggaccatga
 961 aaaggagcag tccatgcaga ctgcagcggg tgagggagtg aaggctgcca atgttggttt
1021 tcagttggat tgcccggagc ttctggacat ggctgtaagt cttgtgaatt tcacaggaaa
1081 aatactggag taaaggaact gttatgttcg cttttgttat atgaaaaccc actgccactt
1141 tatttacagt aaagtgtgtt taagttaaaa tttaaaggaa tatgctgttt ttcacatgta
1201 tattgagtgt gagttttgtg cttcttatta taagtcctgt gtctgatgct gatgaatcac
1261 catctcctga ttctactacc tcacctcctg atattcaagc acctgttcct gtggacgtgc
1321 gcaagcccat tcctgtgaag cttaagcctg ggaaacgtcc agcagtggag aaacttgagg
1381 acttgttaca gggtggggac ggacctttgg acttgagtac acggaaacgt ccaagacaat
1441 aagtgttcca tatccgtgtt tacttaaggt gacgtcaata tttgtgtgag agtgcaatgt
1501 aataaaaata tgttaactgt tcactggttt ttattgcttt ttgggcgggg actcaggtat
1561 ataagtagaa gcagacctgt gtggttagct cataggagct ggctttcatc catggaggtt
1621 tgggccattt tggaagacct taggaagact aggcaactgt tagagagcgc ttcggacgga
1681 gtctccggtt tttggagatt ctggttcgct agtgaattag ctagggtagt ttttaggata
1741 aaacaggact ataaacaaga atttgaaaag ttgttggtag attgcccagg actttttgaa
1801 gctcttaatt tgggccatca ggttcacttt aaagaaaaag ttttatcagt tttagacttt
1861 tcaaccccag gtagaactgc tgctgctgtg gcttttctta cttttatatt agataaatgg
1921 atcccgcaga ctcatttcag caggggatac gttttggatt tcatagccac agcattgtgg
1981 agaacatgga aggttcgcaa gatgaggaca atcttaggtt actggccagt gcagcctttg
2041 ggtgtagcgg gaatcctgag gcatccaccg gtcatgccag cggttctgga ggaggaacag
2101 caagaggaca acccgagagc cggcctggac cctccagtgg aggaggcgga gtagctgact
2161 tgtctcctga actgcaacgg gtgcttactg gatctacgtc cactggacgg gatagggggcg
2221 ttaagaggga gagggcatcc agtggtactg atgctagatc tgagttggct ttaagtttaa
2281 tgagtcgcag acgtcctgaa accatttggt ggcatgaggt tcagaaagag ggaagggatg
2341 aagtttctgt attgcaggag aaatattcac tggaacaggt gaaaacatgt tggttggagc
2401 cagaggatga ttgggaggtg gccattaaaa attatgccaa gatagctttg aggcctgata
2461 aacagtataa gatcagtaga cggattaata tccggaatgc ttgttacata tctggaaatg
2521 gggctgaggt ggtaatagat actcaagaca agacagttat tagatgctgc atgatggata
2581 tgtggcctgg agtagtcggt atggaagcag tcacttttgt aaatgttaag tttaggggag
2641 atggttataa tggaatagtg tttatggcca ataccaaact tatattgcat ggttgtagct
2701 tttttggttt caacaatacc tgtgtagatg cctggggaca ggttagtgta cgggggtgta
2761 gtttctatgc gtgttggatt gccacagctg gcagaaccaa gagtcaattg tctctgaaga
2821 aatgcatatt ccaaagatgt aacctgggca ttctgaatga aggcgaagca agggtccgtc
2881 actgcgcttc tacagatact ggatgtttta ttttaattaa gggaaatgcc agcgtaaagc
2941 ataacatgat ttgtggtgct tccgatgaga ggccttatca aatgctcact tgtgctggtg
3001 ggcattgtaa tatgctggct actgtgcata ttgtttccca tcaacgcaaa aaatggcctg
3061 tttttgatca caatgtgttg accaagtgca ccatgcatgc aggtgggcgt agaggaatgt
3121 ttatgcctta ccagtgtaac atgaatcatg tgaaagtgtt gttggaacca gatgcctttt
3181 ccagaatgag cctaacagga atctttgaca tgaacacgca aatctggaag atcctgaggt
3241 atgatgatac gagatcgagg gtgcgcgcat gcgaatgcgg aggcaagcat gccaggttcc
3301 agccggtgtg tgtagatgtg accgaagatc tcagaccgga tcatttggtt attgcccgca
3361 ctggagcaga gttcggatcc agtggagaag aaactgacta aggtgagtat tgggaaaact
3421 ttggggtggg attttcagat ggacagattg agtaaaaatt tgtttttttct gtcttgcagc
3481 tgacatgagt ggaaatgctt cttttaaggg gggagtcttc agcccttatc tgacagggcg
3541 tctcccatcc tgggcaggag ttcgtcagaa tgttatggga tctactgtgg atggaagacc
3601 cgttcaaccc gccaattctt caacgctgac ctatgctact ttaagttctt caccttttgga
3661 cgcagctgca gccgctgccg ccgcctctgt cgccgctaac actgtgcttg gaatgggtta
3721 ctatggaagc atcgtggcta attccacttc ctctaataac ccttctacac tgactcagga
```

FIG. 2A-1

```
3781 caagttactt gtcctttgg cccagctgga ggctttgacc caacgtctgg gtgaactttc
3841 tcagcaggtg gccgagttgc gagtacaaac tgagtctgct gtcggcacgg caaagtctaa
3901 ataaaaaaaa ttccagaatc aatgaataaa taaacgagct tgttgttgat ttaaaatcaa
3961 gtgtttttat ttcatttttc gcgcacggta tgccctggac caccgatctc gatcattgag
4021 aactcggtgg attttttcca gaatcctata gaggtgggat tgaatgttta gatacatggg
4081 cattaggccg tctttggggt ggagatagct ccattgaagg gattcatgct ccggggtagt
4141 gttgtaaatc acccagtcat aacaaggtcg cagtgcatgg tgttgcacaa tatcttttag
4201 aagtaggctg attgccacag ataagccctt ggtgtaggtg tttacaaacc ggttgagctg
4261 ggaggggtgc attcgaggtg aaattatgtg cattttggat tggattttta agttggcaat
4321 attgccgcca agatcccgtc ttgggttcat gttatgaagg actaccaaga cggtgtatcc
4381 ggtacattta ggaaatttat cgtgcagctt ggatggaaaa gcgtggaaaa atttggagac
4441 acccttgtgt cctccgagat tttccatgca ctcatccatg ataatagcaa tggggccgtg
4501 ggcagcggcg cgggcaaaca cgttccgtgg gtctgacaca tcatagttat gttcctgagt
4561 taaatcatca taagccattt taatgaattt ggggcggagc gtaccagatt ggggtatgaa
4621 tgttccttcg ggccccggag catagttccc ctcacagatt tgcatttccc aagctttcag
4681 ttctgagggt ggaatcatgt ccacctgggg ggctatgaag aacaccgttt cgggggcggg
4741 ggtgattagt tgggatgata gcaagtttct gagcaattga gatttgccac atccggtggg
4801 gccataaata attccgatta caggttgcag gtggtagttt agggaacggc aactgccgtc
4861 ttctcgaagc aaggggggca cctcgttcat catttccctt acatgcatat tttcccgcac
4921 caaatccatt aggaggcgct ctcctcctag tgatagaagt tcttgtagtg aggaaaagtt
4981 tttcagcggt tttagaccgt cagccatggg cattttggaa agagtttgct gcaaaagttc
5041 tagtctgttc cacagttcag tgatgtgttc tatggcatct cgatccagca gacctcctcg
5101 tttcgcgggt ttggacggct cctggagtag ggtatgagac gatgggcgtc cagcgctgcc
5161 agggttcggt ccttccaggg tctcagtgtt cgagtcaggg ttgtttccgt cacagtgaag
5221 gggtgtgcgc ctgcttgggc gcttgccagg gtgcgcttca gactcattct gctggtggag
5281 aacttctgtc gcttggcgcc ctgtatgtcg gccaagtagc agtttaccat gagttcgtag
5341 ttgagcgcct cggctgcgtg gcctttggcg cggagcttac ctttggaagt tttcttgcat
5401 accgggcagt ataggcattt cagcgcatac agcttgggcg caaggaaaat ggattctggg
5461 gagtatgcat ccgcgccgca ggaggcgcaa acagtttcac attccaccag ccaggttaaa
5521 tccggttcat tggggtcaaa aacaagtttt ccgccatatt ttttgatgcg tttcttacct
5581 ttggtctcca taagttcgtg tcctcgttga gtgacaaaca ggctgtccgt atctccgtag
5641 actgatttta caggcctctt ctccagtgga gtgcctcggt cttcttcgta caggaactct
5701 gaccactctg atacaaaggc gcgcgtccag gccagcacaa aggaggctat gtgggagggg
5761 tagcgatcgt tgtcaaccag ggggtccacc ttttccaaag tatgcaaaca catgtcaccc
5821 tcttcaacat ccaggaatgt gattggcttg taggtgtatt tcacgtgacc tggggtcccc
5881 gctgggggggg tataaaaggg ggcggttctt tgctcttcct cactgtcttc cggatcgctg
5941 tccaggaacg tcagctgttg gggtaggtat tccctctcga aggcgggcat gacctctgca
6001 ctcaggttgt cagtttctaa gaacgaggag gatttgatat tgacagtgcc ggttgagatg
6061 cctttcatga ggttttcgtc catttggtca gaaaacacaa ttttttttatt gtcaagtttg
6121 gtggcaaatg atccatacag ggcgttggat aaaagtttgg caatggatcg catggtttgg
6181 ttcttttcct tgtccgcgcg ctctttggcg gcgatgttga gttggacata ctcgcgtgcc
6241 aggcacttcc attcggggaa gatagttgtt aattcatctg gcacgattct cacttgccac
6301 cctcgattat gcaaggtaat taaatccaca ctggtggcca cctcgcctcg aaggggttca
6361 ttggtccaac agagcctacc tcctttccta gaacagaaag ggggaagtgg gtctagcata
6421 agttcatcgg gagggtctgc atccatggta aagattcccg gaagtaaatc cttatcaaaa
6481 tagctgatgg gagtggggtc atctaaggcc atttgccatt ctcgagctgc cagtgcgcgc
6541 tcatatgggt taaggggact gccccagggc atgggatggg tgagagcaga ggcatacatg
6601 ccacagatgt catagacgta gatgggatcc tcaaagatgc ctatgtaggt tggatagcat
6661 cgccccctc tgatacttgc tcgcacatag tcatatagtt catgtgatgg cgctagcagc
6721 cccggaccca agttggtgcg attgggtttt tctgttctgt agacgatctg gcgaaagatg
6781 gcgtgagaat tggaagagat ggtgggtctt tgaaaaatgt tgaaatgggc atgaggtaga
6841 cctacagagt ctctgacaaa gtgggcataa gattcttgaa gcttggttac cagttcggcg
6901 gtgacaagta cgtctagggc gcagtagtca agtgtttctt gaatgatgtc ataacctggt
6961 tggtttttct tttcccacag ttcgcggttg agaaggtatt cttcgcgatc cttccagtac
7021 tcttctagcg gaaacccgtc tttgtctgca cggtaagatc ctagcatgta gaactgatta
7081 actgccttgt aagggcagca gcccttctct acgggtagag agtatgcttg agcagctttt
7141 cgtagcgaag cgtgagtaag ggcaaaggtg tctctgacca tgactttgag aaattggtat
7201 ttgaagtcca tgtcgtcaca ggctccctgt tcccagagtt ggaagtctac ccgtttcttg
7261 taggcggggt tgggcaaagc gaaagtaaca tcattgaaga gaatcttacc ggctctgggc
7321 ataaaattgc gagtgatgcg gaaaggctgt ggtacttccg ctcgattgtt gatcacctgg
7381 gcagctagga cgatttcgtc gaaaccgttg atgttgtgtc ctacgatgta taattctatg
7441 aaacgcggcg tgcctctgac gtgaggtagc ttactgagct catcaaaggt taggtctgtg
7501 gggtcagata aggcgtagtg ttcgagagcc cattcgtgca ggtgaggatt tgcatgtagg
```

FIG. 2A-2

```
7561 aatgatgacc aaagatctac cgccagtgct gtttgtaact ggtcccgata ctgacgaaaa
7621 tgccggccaa ttgccatttt ttctggagtg acacagtaga aggttctggg gtcttgttgc
7681 catcgatccc acttgagttt aatggctaga tcgtgggcca tgttgacgag acgctcttct
7741 cctgagagtt tcatgaccag catgaaagga actagttgtt tgccaaagga tcccatccag
7801 gtgtaagttt ccacatcgta ggtcaggaag agtctttctg tgcgaggatg agagccgatc
7861 gggaagaact ggatttcctg ccaccagttg gaggattggc tgttgatgtg atggaagtag
7921 aagtttctgc ggcgcgccga gcattcgtgt ttgtgcttgt acagacggcc gcagtagtcg
7981 cagcgttgca cgggttgtat ctcgtgaatg agctgtacct ggcttccctt gacgagaaat
8041 ttcagtggga agccgaggcc tggcgattgt atctcgtgct cttctatatt cgctgtatcg
8101 gcctgttcat cttctgtttc gatggtggtc atgctgacga gcccccgcgg gaggcaagtc
8161 cagacctcgg cgcgggaggg gcggagctga aggacgagag cgcgcaggct ggagctgtcc
8221 agagtcctga gacgctgcgg actcaggtta gtaggtaggg acagaagatt aacttgcatg
8281 atcttttcca gggcgtgcgg gaggttcaga tggtacttga tttccacagg ttcgtttgta
8341 gagacgtcaa tggcttgcag ggttccgtgt cctttgggcg ccactaccgt acctttgttt
8401 tttcttttga tcggtggtgg ctctcttgct tcttgcatgc tcagaagcgg tgacggggac
8461 gcgcgccggg cggcagcggt tgttccggac ccgggggcat ggctggtagt ggcacgtcgg
8521 cgccgcgcac gggcaggttc tggtattgcg ctctgagaag acttgcgtgc gccaccacgc
8581 gtcgattgac gtcttgtatc tgacgtctct gggtgaaagc taccggcccc gtgagcttga
8641 acctgaaaga gagttcaaca gaatcaattt cggtatcgtt aacggcagct tgtctcagta
8701 tttcttgtac gtcaccagag ttgtcctggt aggcgatctc cgccatgaac tgctcgattt
8761 cttcctcctg aagatctccg cgacccgctc tttcgacggt ggccgcgagg tcattggaga
8821 tacggcccat gagttgggag aatgcattca tgcccgcctc gttccagacg cggctgtaaa
8881 ccacggcccc ctcggagtct cttgcgcgca tcaccacctg agcgaggtta agctccacgt
8941 gtctggtgaa gaccgcatag ttgcataggc gctgaaaaag gtagttgagt gtggtggcaa
9001 tgtgttcggc gacgaagaaa tacatgatcc atcgtctcag cggcatttcg ctaacatcgc
9061 ccagagcttc caagcgctcc atggcctcgt agaagtccac ggcaaaatta aaaaactggg
9121 agtttcgcgc ggacacggtc aattcctcct cgagaagacg gatgagttcg gctatggtgg
9181 cccgtacttc gcgttcgaag gctcccggga tctcttcttc ctcttctatc tcttcttcca
9241 ctaacatctc ttcttcgtct tcaggcgggg gcggaggggg cacgcggcga cgtcgacggc
9301 gcacgggcaa acggtcgatg aatcgttcaa tgacctctcc gcggcggcgg cgcatggttt
9361 cagtgacggc gcggccgttc tcgcgcggtc gcagagtaaa aacaccgccg cgcatctcct
9421 taaagtggtg actgggaggt tctccgtttg ggagggagag ggcgctgatt atacatttta
9481 ttaattggcc cgtagggact gcgcgcagag atctgatcgt gtcaagatcc acgggatctg
9541 aaaacctttc gacgaaagcg tctaaccagt cacagtcaca aggtaggctg agtacggctt
9601 cttgtgggcg ggggtggtta tgtgttcggt ctgggtcttc tgtttcttct tcatctcggg
9661 aaggtgagac gatgctgctg gtgatgaaat taaagtaggc agttctaaga cggcggatgg
9721 tggcgaggag caccaggtct ttgggtccgg cttgctggat acgcaggcga ttggccattc
9781 cccaagcatt atcctgacat ctagcaagat ctttgtagta gtcttgcatg agccgttcta
9841 cgggcacttc ttcctcaccc gttctgccat gcatacgtgt gagtccaaat ccgcgcattg
9901 gttgtaccag tgccaagtca gctacgactc tttcggcgag gatggcttgc tgtacttggg
9961 taagggtggc ttgaaagtca tcaaaatcca caaagcggtg gtaagcccct gtattaatgg
10021 tgtaagcaca gttggccatg actgaccagt taactgtctg gtgaccaggg cgcacgagct
10081 cggtgtattt aaggcgcgaa taggcgcggg tgtcaaagat gtaatcgttg caggtgcgca
10141 ccagatactg gtaccctata agaaaatgcg gcggtggttg gcggtagaga ggccatcgtt
10201 ctgtagctgg agcgccaggg gcgaggtctt ccaacataag gcggtgatag ccgtagatgt
10261 acctggacat ccaggtgatt cctgcggcgg tagtagaagc ccgaggaaac tcgcgtacgc
10321 ggttccaaat gttgcgtagc ggcatgaagt agttcattgt aggcacggtt tgaccagtga
10381 ggcgcgcgca gtcattgatg ctctatagac acggagaaaa tgaaagcgtt cagcgactcg
10441 actccgtagc ctggaggaac gtgaacgggt tgggtcgcgg tgtaccccgg ttcgagactt
10501 gtactcgagc cggccggagc cgcggctaac gtggtattgg cactcccgtc tcgacccagc
10561 ctacaaaaat ccaggatacg gaatcgagtc gttttgctgg tttccgaatg gcagggaagt
10621 gagtcctatt ttttttttct ttttgccgct cagatgcatc ccgtgctgcg acagatgcgc
10681 ccccaacaac agccccccctc gcagcagcag cagcagcagc aaccacaaaa ggctgtccct
10741 gcaactactg caactgccgc cgtgagcggt gcgggacagc ccgcctatga tctggacttg
10801 gaagagggcg aaggactggc acgtctaggt gcgccttcgc ccgagcggca tccgcgagtt
10861 caactgaaaa aagattctcg cgaggcgtat gtgccccaac agaacctatt tagagacaga
10921 agcggcgagg agccggagga gatgcgagct tcccgcttta acgcgggtcg tgagctgcgt
10981 cacggtttgg accgaagacg agtgttgcga gacgaggatt tcgaagttga tgaagtgaca
11041 gggatcagtc ctgccagggc acacgtggct gcagccaacc ttgtatcggc ttacgagcag
11101 acagtaaagg aagagcgtaa cttccaaaag tcttttaata atcatgtgcg aaccctgatt
11161 gcccgcgaag aagttaccct tggtttgatg catttgtggg atttgatgga agctatcatt
11221 cagaacccta ctagcaaacc tctgaccgcc cagctgtttc tggtggtgca acacagcaga
11281 gacaatgagg ctttcagaga ggcgctgctg aacatcaccg aacccgaggg gagatggttg
```

FIG. 2A-3

```
11341 tatgatctta tcaacattct acagagtatc atagtgcagg agcggagcct gggcctggcc
11401 gagaaggtag ctgccatcaa ttactcggtt ttgagcttgg gaaaatatta cgctcgcaaa
11461 atctacaaga ctccatacgt tcccatagac aaggaggtga agatagatgg gttctacatg
11521 cgcatgacgc tcaaggtctt gaccctgagc gatgatcttg gggtgtatcg caatgacaga
11581 atgcatcgcg cggttagcgc cagcaggagg cgcgagttaa gcgacaggga actgatgcac
11641 agtttgcaaa gagctctgac tggagctgga accgagggtg agaattactt cgacatggga
11701 gctgacttgc agtggcagcc tagtcgcagg gctctgagcg ccgcgacggc aggatgtgag
11761 cttccttaca tagaagaggc ggatgaaggc gaggaggaag agggcgagta cttggaagac
11821 tgatggcaca acccgtgttt tttgctagat ggaacagcaa gcaccggatc ccgcaatgcg
11881 ggcggcgctg cagagccagc cgtccggcat taactcctcg gacgattgga cccaggccat
11941 gcaacgtatc atggcgttga cgactcgcaa ccccgaagcc tttagacagc aaccccaggc
12001 caaccgtcta tcggccatca tggaagctgt agtgccttcc cgatctaatc ccactcatga
12061 gaaggtcctg gccatcgtga acgcgttggt ggagaacaaa gctattcgtc cagatgaggc
12121 cggactggta tacaacgctc tcttagaacg cgtggctcgc tacaacagta gcaatgtgca
12181 aaccaatttg gaccgtatga taacagatgt acgcgaagcc gtgtctcagc gcgaaaggtt
12241 ccagcgtgat gccaacctgg gttcgctggt ggcgttaaat gctttcttga gtactcagcc
12301 tgctaatgtg ccgcgtggtc aacaggatta tactaacttt ttaagtgctt tgagactgat
12361 ggtatcagaa gtacctcaga gcgaagtgta tcagtccggt cctgattact tctttcagac
12421 tagcagacag ggcttgcaga cggtaaatct gagccaagct tttaaaaacc ttaaaggttt
12481 gtggggagtg catgccccgg taggagaaag agcaaccgtg tctagcttgt taactccgaa
12541 ctcccgcctg ttattactgt tggtagctcc tttcaccgac agcggtagca tcgaccgtaa
12601 ttcctatttg ggttacctac taaacctgta tcgcgaagcc atagggcaaa gtcaggtgga
12661 cgagcagacc tatcaagaaa ttacccaagt cagtcgcgct ttgggacagg aagacactgg
12721 cagtttggaa gccactctga acttcttgct taccaatcgg tctcaaaaga tccctcctca
12781 atatgctctt actgcggagg aggagaggat ccttagatat gtgcagcaga gcgtgggatt
12841 gtttctgatg caagaggggg caactccgac tgcagcactg gacatgacag cgcgaaatat
12901 ggagcccagc atgtatgcca gtaaccgacc tttcattaac aaactgctgg actacttgca
12961 cagagctgcc gctatgaact ctgattattt caccaatgcc atcttaaacc cgcactggct
13021 gcccccacct ggtttctaca cgggcgaata tgacatgccc gaccctaatg acggatttct
13081 gtgggacgac gtggacagcg atgtttttc acctctttct gatcatcgca cgtggaaaaa
13141 ggaaggcggt gatagaatgc attcttctgc atcgctgtcc ggggtcatgg gtgctaccgc
13201 ggctgagccc gagtctgcaa gtccttttcc tagtctaccc ttttctctac acagtgtacg
13261 tagcagcgaa gtgggtagaa taagtcgccc gagtttaatg ggcgaagagg agtacctaaa
13321 cgattccttg ctcagaccgg caagagaaaa aaatttccca aacaatggaa tagaaagttt
13381 ggtggataaa atgagtagat ggaagactta tgctcaggat cacagagacg agcctgggat
13441 catggggact acaagtagag cgagccgtag acgccagcgc catgacagac agaggggtct
13501 tgtgtgggac gatgaggatt cggccgatga tagcagcgtg ttggacttgg gtgggagagg
13561 aaggggcaac ccgtttgctc atttgcgccc tcgcttgggt ggtatgttgt gaaaaaaaat
13621 aaaaaagaaa aactcaccaa ggccatggcg acgagcgtac gttcgttctt ctttattatc
13681 tgtgtctagt ataatgaggc gagtcgtgct aggcggagcg gtggtgtatc cggagggtcc
13741 tcctccttcg tacgagagcg tgatgcagca gcagcaggcg acggcggtga tgcaatcccc
13801 actggaggct ccctttgtgc ctccgcgata cctggcacct acggagggca gaaacagcat
13861 tcgttactcg gaactggcac ctcagtacga taccaccagg ttgtatctgg tggacaacaa
13921 gtcggcggac attgcttctc tgaactatca gaatgaccac agcaacttct tgaccacggt
13981 ggtgcagaac aatgacttta cccctacgga agccagcacc cagaccatta actttgatga
14041 acgatcgcgg tggggcggtc agctaaagac catcatgcat actaacatgc caaacgtgaa
14101 cgagtatatg tttagtaaca agttcaaagc gcgtgtgatg gtgtccagaa aacctcccga
14161 cggtgctgca gttggggata cttatgatca caagcaggat attttggaat atgagtggtt
14221 cgagtttact ttgccagaag gcaacttttc agttactatg actattgatt tgatgaacaa
14281 tgccatcata gataattact tgaaagtggg tagacagaat ggagtgcttg aaagtgacat
14341 tggtgttaag ttcgacacca ggaacttcaa gctgggatgg gatcccgaaa ccaagttgat
14401 catgcctgga gtgtatacgt atgaagcctt ccatcctgac attgtcttac tgcctggctg
14461 cggagtggat tttaccgaga gtcgtttgag caaccttctt ggtatcagaa aaaaacagcc
14521 atttcaagag ggttttaaga ttttgtatga agatttagaa ggtggtaata ttccggccct
14581 cttggatgta gatgcctatg agaacagtaa gaaagaacaa aaagccaaaa tagaagctgc
14641 tacagctgct gcagaagcta aggcaaacat agttgccagc gactctacaa gggttgctaa
14701 cgctggagag gtcagaggag acaattttgc gccaacacct gttccgactg cagaatcatt
14761 attggccgat gtgtctgatg gaacggacgt gaaactcact attcaacctg tagaaaaaga
14821 tagtaagaat agaagctata atgtgttgga agacaaaatc aacacagcct atcgcagttg
14881 gtatctttcg tacaattatg gcgatcccga aaaaggagtg cgttcctgga cattgctcac
14941 cacctcagat gtcacctgcg gagcagagca ggtttactgg tcgcttccag acatgatgaa
15001 ggatcctgtc actttccgct ccactagaca agtcagtaac taccctgtgg tgggtgcaga
15061 gcttatgccc gtcttctcaa agagcttcta caacgaacaa gctgtgtact cccagcagct
```

FIG. 2A-4

15121 ccgccagtcc acctcgctta cgcacgtctt caaccgcttt cctgagaacc agattttaat
15181 ccgtccgccg gcgcccacca ttaccaccgt cagtgaaaac gttcctgctc tcacagatca
15241 cgggaccctg ccgttgcgca gcagtatccg gggagtccaa cgtgtgaccg ttactgacgc
15301 cagacgccgc acctgtccct acgtgtacaa ggcactgggc atagtcgcac cgcgcgtcct
15361 ttcaagccgc actttctaaa aaaaaaatgt ccattcttat ctcgcccagt aataacaccg
15421 gttggggtct gcgcgctcca agcaagatgt acggaggcgc acgcaaacgt tctacccaac
15481 atcccgtgcg tgttcgcgga cattttcgcg ctccatgggg tgccctcaag ggccgcactc
15541 gcgttcgaac caccgtcgat gatgtaatcg atcaggtggt tgccgacgcc cgtaattata
15601 ctcctactgc gcctacatct actgtggatg cagttattga cagtgtagtg gctgacgctc
15661 gcaactatgc tcgacgtaag agccggcgaa ggcgcattgc cagacgccac cgagctacca
15721 ctgccatgcg agccgcaaga gctctgctac gaagagctag acgcgtgggg cgaagagcca
15781 tgcttagggc ggccagacgt gcagcttcgg gcgccagcgc cggcaggtcc cgcaggcaag
15841 cagccgctgt cgcagcggcg actattgccg acatggccca atcgcgaaga ggcaatgtat
15901 actgggtgcg tgacgctgcc accggtcaac gtgtacccgt gcgcacccgt ccccctcgca
15961 cttagaagat actgagcagt ctccgatgtt gtgtcccagc ggcgaggatg tccaagcgca
16021 aatacaagga agaaatgctg caggttatcg cacctgaagt ctacggccaa ccgttgaagg
16081 atgaaaaaaa accccgcaaa atcaagcggg ttaaaaagga caaaaaagaa gaggaagatg
16141 gcgatgatgg gctggcggag tttgtgcgcg agtttgcccc acggcgacgc gtgcaatggc
16201 gtgggcgcaa agttcgacat gtgttgagac ctggaacttc ggtggtcttt acacccggcg
16261 agcgttcaag cgctactttt aagcgttcct atgatgaggt gtacggggat gatgatattc
16321 ttgagcaggc ggctgaccga ttaggcgagt ttgcttatgg caagcgtagt agaataactt
16381 ccaaggatga gacagtgtca ataccttgg atcatggaaa tcccacccct agtcttaaac
16441 cggtcacttt gcagcaagtg ttacccgtaa ctccgcgaac aggtgttaaa cgcgaaggtg
16501 aagatttgta tcccactatg caactgatgg tacccaaacg ccagaagttg gaggacgttt
16561 tggagaaagt aaaagtggat ccagatattc aacctgaggt taaagtgaga cccattaagc
16621 aggtagcgcc tggtctgggg gtacaaactg tagacattaa gattcccact gaaagtatgg
16681 aagtgcaaac tgaacccgca aagcctactg ccacctccac tgaagtgcaa acggatccat
16741 ggatgcccat gcctattaca actgacgccg ccggtcccac tcgaagatcc cgacgaaagt
16801 acggtccagc aagtctgttg atgcccaatt atgttgtaca cccatctatt attcctactc
16861 ctggttaccg aggcactcgc tactatcgca gccgaaacag tacctcccgc cgtcgccgca
16921 agacacctgc aaatcgcagt cgtcgccgta gacgcacaag caaaccgact cccggcgccc
16981 tggtgcggca agtgtaccgc aatggtagtg cggaaccttt gacactgccg cgtgcgcgtt
17041 accatccgag tatcatcact taatcaatgt tgccgctgcc tccttgcaga tatggccctc
17101 acttgtcgcc ttcgcgttcc catcactggt taccgaggaa gaaactcgcg ccgtagaaga
17161 gggatgttgg gacgcggaat gcgacgctac aggcgacggc gtgctatccg caagcaattg
17221 cggggtggtt ttttaccagc cttaattcca attatcgctg ctgcaattgg cgcgatacca
17281 ggcatagctt ccgtggcggt tcaggcctcg caacgacatt gacattggaa aaaaaacgta
17341 taaataaaaa aaaatacaat ggactctgac actcctggtc ctgtgactat gttttcttag
17401 agatggaaga catcaatttt tcatccttgg ctccgcgaca cggcacgaag ccgtacatgg
17461 gcacctggag cgacatcggc acgagccaac tgaacggggg cgccttcaat tggagcagta
17521 tctggagcgg gcttaaaaat tttggctcaa ccataaaaac atacgggaac aaagcttgga
17581 acagcagtac aggacaggcg cttagaaata aacttaaaga ccagaacttc caacaaaaag
17641 tagtcgatgg gatagcttcc ggcatcaatg gagtggtaga tttggctaac caggctgtgc
17701 agaaaaagat aaacagtcgt ttggacccgc cgccagcaac cccaggtgaa atgcaagtgg
17761 aggaagaaat tcctccgcca gaaaaacgag gcgacaagcg tccgcgtccc gatttggaag
17821 agacgctggt gacgcgcgta gatgaaccgc cttcttatga ggaagcaacg aagcttggaa
17881 tgcccaccac tagaccgata gccccaatgg ccaccggggt gatgaaacct tctcagttgc
17941 atcgacccgt caccttggat ttgccccctc cccctgctgc tactgctgta cccgcttcta
18001 agcctgtcgc tgccccgaaa ccagtcgccg tagccaggtc acgtcccggg ggcgctcctc
18061 gtccaaatgc gcactggcaa aatactctga acagcatcgt gggtctaggc gtgcaaagtg
18121 taaaacgccg tcgctgcttt taattaaata tggagtagcg cttaacttgc ctatctgtgt
18181 atatgtgtca ttacacgccg tcacagcagc agaggaaaaa aggaagaggt cgtgcgtcga
18241 cgctgagtta ctttcaagat ggccacccca tcgatgctgc cccaatgggc atacatgcac
18301 atcgccggac aggatgcttc ggagtacctg agtccgggtc tggtgcagtt cgcccgcgcc
18361 acagacacct acttcaatct gggaaataag tttagaaatc ccaccgtagc gccgacccac
18421 gatgtgacca ccgaccgtag ccagcggctc atgttgcgct tcgtgcccgt tgaccgggag
18481 gacaatacat actcttacaa agtgcggtac accctggccg tgggcgacaa cagagtgctg
18541 gatatggcca gcacgttctt tgacattagg ggcgtgttgg acagaggtcc cagtttcaaa
18601 ccctattctg gtacggctta caactctctg gctcctaaag gcgctccaaa tgcatctcaa
18661 tggattgcaa aaggcgtacc aactgcagca gccgcaggca atggtgaaga agaacatgaa
18721 acagaggaga aaactgctac ttacactttt gccaatgctc ctgtaaaagc cgaggctcaa
18781 attacaaaag agggcttacc aataggtttg gagatttcag ctgaaaacga atctaaaccc
18841 atctatgcag ataaacttta tcagccagaa cctcaagtgg gagatgaaac ttggactgac

FIG. 2A-5

```
18901 ctagacggaa aaaccgaaga gtatggaggc agggctctaa agcctactac taacatgaaa
18961 ccctgttacg ggtcctatgc gaagcctact aatttaaaag gtggtcaggc aaaaccgaaa
19021 aactcggaac cgtcgagtga aaaaattgaa tatgatattg acatggaatt ttttgataac
19081 tcatcgcaaa gaacaaactt cagtcctaaa attgtcatgt atgcagaaaa tgtaggtttg
19141 gaaacgccag acactcatgt agtgtacaaa cctggaacag aagacacaag ttccgaagct
19201 aatttgggac aacagtctat gcccaacaga cccaactaca ttggcttcag agataacttt
19261 attggactca tgtactataa cagtactggt aacatggggg tgctggctgg tcaagcgtct
19321 cagttaaatg cagtggttga cttgcaggac agaaacacag aactttctta ccaactcttg
19381 cttgactctc tgggcgacag aaccagatac tttagcatgt ggaatcaggc tgtggacagt
19441 tatgatcctg atgtacgtgt tattgaaaat catggtgtgg aagatgaact tcccaactat
19501 tgttttccac tggacggcat aggtgttcca acaaccagtt acaaatcaat agttccaaat
19561 ggagaagata ataataattg gaaagaacct gaagtaaatg gaacaagtga gatcggacag
19621 ggtaatttgt ttgccatgga aattaacctt caagccaatc tatggcgaag tttcctttat
19681 tccaatgtgg ctctgtatct cccagactcg tacaaataca ccccgtccaa tgtcactctt
19741 ccagaaaaca aaaacaccta cgactacatg aacgggcggg tggtgccgcc atctctagta
19801 gacacctatg tgaacattgg tgccaggtgg tctctggatg ccatggacaa tgtcaaccca
19861 ttcaaccacc accgtaacgc tggcttgcgt taccgatcta tgcttctggg taacggacgt
19921 tatgtgcctt tccacataca agtgcctcaa aaattcttcg ctgttaaaaa cctgctgctt
19981 ctcccaggct cctacactta tgagtggaac tttaggaagg atgtgaacat ggttctacag
20041 agttccctcg gtaacgacct gcgggtagat ggcgccagca tcagtttcac gagcatcaac
20101 ctctatgcta ctttttttccc catggctcac aacaccgctt ccacccttga agccatgctg
20161 cggaatgaca ccaatgatca gtcattcaac gactacctat ctgcagctaa catgctctac
20221 cccattcctg ccaatgcaac caatattccc atttccattc cttctcgcaa ctgggcggct
20281 ttcagaggct ggtcatttac cagactgaaa accaaagaaa ctcccctctt ggggtctgga
20341 tttgacccct actttgtcta ttctggttct attccctacc tggatggtac cttctacctg
20401 aaccacactt ttaagaaggt ttccatcatg tttgactctt cagtgagctg gcctggaaat
20461 gacaggttac tatctcctaa cgaatttgaa ataaagcgca ctgtggatgg cgaaggctac
20521 aacgtagccc aatgcaacat gaccaaagac tggttcttgg tacagatgct cgccaactac
20581 aacatcggct atcagggctt ctacattcca gaaggataca aagatcgcat gtattcattt
20641 ttcagaaact tccagcccat gagcaggcag gtggttgatg aggtcaatta caaagacttc
20701 aaggccgtcg ccatacccta ccaacacaac aactctggct ttgtgggtta catggctccg
20761 accatgcgcc aaggtcaacc ctatcccgct aactatccct atccactcat tggaacaact
20821 gccgtaaata gtgttacgca gaaaaagttc ttgtgtgaca gaaccatgtg gcgcataccg
20881 ttctcgagca acttcatgtc tatgggggcc cttacagact tgggacagaa tatgctctat
20941 gccaactcag ctcatgctct ggacatgacc tttgaggtgg atcccatgga tgagcccacc
21001 ctgctttatc ttctcttcga agttttcgac gtggtcagag tgcatcagcc acaccgcggc
21061 atcatcgagg cagtctacct gcgtacaccg ttctcggccg gtaacgctac cacgtaagaa
21121 gcttcttgct tcttgcaaat agcagctgca accatggcct gcggatccca aaacggctcc
21181 agcgagcaag agctcagagc cattgtccaa gacctgggtt gcggacccta ttttttggga
21241 acctacgata agcgcttccc ggggttcatg gcccccgata agctcgcctg tgccattgta
21301 aatacggccg gacgtgagac ggggggagag cactggttgg ctttcggttg gaacccacgt
21361 tctaacacct gctacctttt tgatcctttt ggattctcgg atgatcgtct caaacagatt
21421 taccagtttg aatatgaggg tctcctgcgc cgcagcgctc ttgctaccaa ggaccgctgt
21481 attacgctgg aaaaatctac ccagaccgtg cagggccccc gttctgccgc ctgcggactt
21541 ttctgctgca tgttccttca cgcctttgtg cactggcctg accgtcccat ggacggaaac
21601 cccaccatga aattgctaac tggagtgcca aacaacatgc ttcattctcc taaagtccag
21661 cccaccctgt gtgacaatca aaaagcactc taccattttc ttaataccca ttcgccttat
21721 tttcgctctc atcgtacaca catcgaaagg gccactgcgt tcgaccgtat ggatgttcaa
21781 taatgactca tgtaaacaac gtgttcaata aacatcactt tatttttta catgtatcaa
21841 ggctctggat tacttattta tttacaagtc gaatgggttc tgacgagaat cagaatgacc
21901 cgcaggcagt gatacgttgc ggaactgata cttgggttgc cacttgaatt cgggaatcac
21961 caacttggga accggtatat cgggcaggat gtcactccac agctttctgg tcagctgcaa
22021 agctccaagc aggtcaggag ccgaaatctt gaaatcacaa ttaggaccag tgctctgagc
22081 gcgagagttg cggtacaccg gattgcagca ctgaaacacc atcagcgacg gatgtctcac
22141 gcttgccagc acggtgggat ctgcaatcat gcccacatcc agatcttcag cattggcaat
22201 gctgaacggg gtcatcttgc aggtctgcct acccatggcg ggcacccaat taggcttgtg
22261 gttgcaatcg cagtgcaggg ggatcagtat catcttggcc tgatcctgtc tgattcctgg
22321 atacacggct ctcatgaaag catcatattg cttgaaagcc tgctgggctt tactaccctc
22381 ggtataaaac atcccgcagg acctgctcga aaactggtta gctgcacagc cggcatcatt
22441 cacacagcag cgggcgtcat tgttggctat ttgcaccaca cttctgcccc agcggttttg
22501 ggtgattttg gttcgctcgg gattctcctt taaggctcgt tgtccgttct cgctggccac
22561 atccatctcg ataatctgct ccttctgaat cataatattg ccatgcaggc acttcagctt
22621 gccctcataa tcattgcagc catgaggcca caacgcacag cctgtacatt cccaattatg
```

FIG. 2A-6

```
22681 gtgggcgatc tgagaaaaag aatgtatcat tccctgcaga aatcttccca tcatcgtgct
22741 cagtgtcttg tgactagtga aagttaactg gatgcctcgg tgctcttcgt ttacgtactg
22801 gtgacagatg cgcttgtatt gttcgtgttg ctcaggcatt agtttaaaac aggttctaag
22861 ttcgttatcc agcctgtact tctccatcag cagacacatc acttccatgc ctttctccca
22921 agcagacacc aggggcaagc taatcggatt cttaacagtg caggcagcag ctcctttagc
22981 cagagggtca tctttagcga tcttctcaat gcttcttttg ccatccttct caacgatgcg
23041 cacgggcggg tagctgaaac ccactgctac aagttgcgcc tcttctcttt cttcttcgct
23101 gtcttgactg atgtcttgca tggggatatg tttggtcttc cttggcttct ttttgggggg
23161 tatcggagga ggaggactgt cgctccgttc cggagacagg gaggattgtg acgtttcgct
23221 caccattacc aactgactgt cggtagaaga acctgacccc acacggcgac aggtgttttt
23281 cttcgggggc agaggtggag gcgattgcga agggctgcgg tccgacctgg aaggcggatg
23341 actggcagaa ccccttccgc gttcgggggt gtgctccctg tggcggtcgc ttaactgatt
23401 tccttcgcgg ctggccattg tgttctccta ggcagagaaa caacagacat ggaaactcag
23461 ccattgctgt caacatcgcc acgagtgcca tcacatctcg tcctcagcga cgaggaaaag
23521 gagcagagct taagcattcc accgcccagt cctgccacca cctctaccct agaagataag
23581 gaggtcgacg catctcatga catgcagaat aaaaaagcga aagagtctga gacagacatc
23641 gagcaagacc cgggctatgt gacaccggtg gaacacgagg aagagttgaa acgctttcta
23701 gagagagagg atgaaaactg cccaaaacag cgagcagata actatcacca agatgctgga
23761 aatagggatc agaacaccga ctacctcata gggcttgacg gggaagacgc gctccttaaa
23821 catctagcaa gacagtcgct catagtcaag gatgcattat tggacagaac tgaagtgccc
23881 atcagtgtgg aagagctcag ctgcgcctac gagcttaacc ttttttcacc tcgtactccc
23941 cccaaacgtc agccaaacgg cacctgcgag ccaaatcctc gcttaaactt ttatccagct
24001 tttgctgtgc cagaagtact ggctacctat cacatctttt ttaaaaatca aaaaattcca
24061 gtctcctgcc gcgctaatcg cacccgcgcc gatgccctac tcaatctggg acctggttca
24121 cgcttacctg atatagcttc cttggaagag gttccaaaga tcttcgaggg tctgggcaat
24181 aatgagactc gggccgcaaa tgctctgcaa aagggagaaa atggcatgga tgagcatcac
24241 agcgttctgg tggaattgga aggcgataat gccagactcg cagtactcaa gcgaagcgtc
24301 gaggtcacac acttcgcata tcccgctgtc aacctgcccc ctaaagtcat gacggcggtc
24361 atggaccagt tactcattaa gcgcgcaagt ccccttcag aagacatgca tgacccagat
24421 gcctgtgatg agggtaaacc agtggtcagt gatgagcagc taacccgatg gctgggcacc
24481 gactctcccc gggatttgga agagcgtcgc aagcttatga tggccgtggt gctggttacc
24541 gtagaactag agtgtctccg acgtttcttt accgattcag aaaccttgcg caaactcgaa
24601 gagaatctgc actacacttt tagacacggc tttgtgcggc aggcatgcaa gatatctaac
24661 gtggaactca ccaacctggt ttcctacatg ggtattctgc atgagaatcg cctaggacaa
24721 agcgtgctgc acagcaccct taaggggaa gcccgccgtg attacatccg cgattgtgtc
24781 tatctctacc tgtgccacac gtggcaaacc ggcatgggtg tatggcagca atgtttagaa
24841 gaacagaact tgaaagagct tgacaagctc ttacagaaat ctcttaaggt tctgtggaca
24901 gggttcgacg agcgcaccgt cgcttccgac ctggcagacc tcatcttccc agagcgtctc
24961 agggttactt tgcgaaacgg attgcctgac tttatgagcc agagcatgct taacaatttt
25021 cgctctttca tcctggaacg ctccggtatc ctgcccgcca cctgctgcgc actgccctcc
25081 gactttgtgc ctctcaccta ccgcgagtgc cccccgccgc tatggagtca ctgctacctg
25141 ttccgtctgg ccaactatct ctcctaccac tcggatgtga tcgaggatgt gagcggagac
25201 ggcttgctgg agtgccactg ccgctgcaat ctgtgcacgc cccaccggtc cctagcttgc
25261 aacccccagt tgatgagcga aacccagata ataggcacct ttgaattgca aggccccagc
25321 agccaaggcg atgggtcttc tcctgggcaa agtttaaaac tgaccccggg actgtggacc
25381 tccgcctact tgcgcaagtt tgctccggaa gattaccacc cctatgaaat caagttctat
25441 gaggaccaat cacagcctcc aaaggccgaa ctttcggctt gcgtcatcac ccaggggca
25501 attctggccc aattgcaagc catccaaaaa tcccgccaag aatttctact gaaaaagggt
25561 aaggggtct accttgaccc ccagaccggc gaggaactca acacaaggtt ccctcaggat
25621 gtcccaacga cgagaaaaca agaagttgaa ggtgcagccg ccgccccag aagatatgga
25681 ggaagattgg gacagtcagg cagaggaggc ggaggaggac agtctggagg acagtctgga
25741 ggaagacagt ttggaggagg aaaacgagga ggcagaggag gtggaagaag taaccgccga
25801 caaacagtta tcctcggctg cggagacaag caacagcgct accatctccg ctccgagtcg
25861 aggaacccgg cggcgtccca gcagtagatg ggacgagacc ggacgcttcc cgaacccaac
25921 cagcgcttcc aagaccggta agaaggatcg gcagggatac aagtcctggc gggggcataa
25981 gaatgccatc atctcctgct tgcatgagtg cgggggcaac atatccttca cgcggcgcta
26041 cttgctattc caccatgggg tgaactttcc gcgcaatgtt ttgcattact accgtcacct
26101 ccacagcccc tactatagcc agcaaatccc gacagtctcg acagataaag acagcggcgg
26161 cgacctccaa cagaaaacca gcagcggcag ttagaaaata cacaacaagt gcagcaacag
26221 gaggattaaa gattacagcc aacgagccag cgcaaacccg agagttaaga aatcggatct
26281 ttccaaccct gtatgccatc ttccagcaga gtcggggtca agagcaggaa ctgaaaataa
26341 aaaaccgatc tctgcgttcg ctcaccagaa gttgtttgta tcacaagagc gaagatcaac
26401 ttcagcgcac tctcgaggac gccgaggctc tcttcaacaa gtactgcgcg ctgactctta
```

FIG. 2A-7

```
26461 aagagtaggc agcgaccgcg cttattcaaa aaaggcggga attacatcat cctcgacatg
26521 agtaaagaaa ttcccacgcc ttacatgtgg agttatcaac cccaaatggg attggcagca
26581 ggcgcctccc aggactactc cacccgcatg aattggctca gcgccgggcc ttctatgatt
26641 tctcgagtta atgatatacg cgcctaccga aaccaaatac ttttggaaca gtcagctctt
26701 accaccacgc cccgccaaca ccttaatccc agaaattggc ccgccgccct agtgtaccag
26761 gaaagtcccg ctcccaccac tgtattactt cctcgagacg cccaggccga agtccaaatg
26821 actaatgcag gtgcgcagtt agctggcggc tccaccctat gtcgtcacag gcctcggcat
26881 aatataaaac gcctgatgat cagaggccga ggtatccagc tcaacgacga gtcggtgagc
26941 tctccgcttg gtctacgacc agacggaatc tttcagattg ccggctgcgg gagatcttcc
27001 ttcacccctc gtcaggctgt tctgactttg gaaagttcgt cttcgcaacc ccgctcgggc
27061 ggaatcggga ccgttcaatt tgtagaggag tttactccct ctgtctactt caacccccttc
27121 tccggatctc ctgggcacta cccggacgag ttcataccga acttcgacgc gattagcgag
27181 tcagtggacg gctacgattg atgtctggtg acgcggctga gctatctcgg ctgcgacatc
27241 tagaccactg ccgccgcttt cgctgctttg cccgggaact tattgagttc atctacttcg
27301 aactccccaa ggatcaccct caaggtccgg cccacggagt gcggattact atcgaaggca
27361 aaatagactc tcgcctgcaa cgaatttttct cccagcggcc cgtgctgatc gagcgagacc
27421 agggaaacac cacggtttcc atctactgca tttgtaatca ccccggattg catgaaagcc
27481 tttgctgtct tatgtgtact gagtttaata aaaactgaat taagactctc ctacggactg
27541 ccgcttcttc aacccggatt ttacaaccag aagaacaaaa cttttcctgt cgtccaggac
27601 tctgttaact tcacctttcc tactcacaaa ctagaagctc aacgactaca ccgcttttcc
27661 agaagcattt tccctactaa tactactttc aaaaccggag gtgagctcca cggtctccct
27721 acagaaaacc cttgggtgga agcgggcctt gtagtactag gaattcttgc gggtgggctt
27781 gtgattattc tttgctacct atacacacct tgcttcactt tcctagtggt gttgtggtat
27841 tggtttaaaa aatggggccc atactagtct tgcttgtttt actttcgctt ttggaaccgg
27901 gttctgccaa ttacgatcca tgtctagact ttgacccaga aaactgcaca cttacttttg
27961 cacccgacac aagccgcatc tgtggagttc ttattaagtg cggatgggaa tgcaggtccg
28021 ttgaaattac acacaataac aaaacctgga acaatacctt atccaccaca tgggagccag
28081 gagttcccga gtggtacact gtctctgtcc gaggtcctga cggttccatc cgcattagta
28141 acaacacttt catttttct gaaatgtgcg atctggccat gttcatgagc aaacagtatt
28201 ctctatggcc tcctagcaag gacaacatcg taacgttctc cattgcttat tgcttgtgcg
28261 cttgccttct tactgcttta ctgtgcgtat gcatacacct gcttgtaacc actcgcatca
28321 aaaacgccaa taacaaagaa aaaatgcctt aacctctttc tgtttacaga catggcttct
28381 cttacatctc tcatatttgt cagcattgtc actgccgctc acggacaaac agtcgtctct
28441 atcccactag gacataatta cactctcata ggacccccaa tcacttcaga ggtcatctgg
28501 accaaactgg gaagcgttga ttactttgat ataatctgta acaaaacaaa accaataata
28561 gtaacttgca acatacaaaa tcttacattg attaatgtta gcaaagttta cagcggttac
28621 tattatggtt atgacagata cagtagtcaa tatagaaatt acttggttcg tgttacccag
28681 ttgaaaacca cgaaaatgcc aaatatggca aagattcgat ccgatgacaa ttctctagaa
28741 acttttacat ctcccaccac acccgacgaa aaaaacatcc cagattcaat gattgcaatt
28801 gttgcagcgg tggcagtggt gatggcacta ataataatat gcatgctttt atatgcttgt
28861 cgctacaaaa agtttcatcc taaaaaacaa gatctcctac taaggcttaa catttaattt
28921 ctttttatac agccatggtt tccactacca cattccttat gcttactagt ctcgcaactc
28981 tgacttctgc tcgctcacac ctcactgtaa ctataggctc aaactgcaca ctaaaaggac
29041 ctcaaggtgg tcatgtcttt tggtggagaa tatatgacaa tggatggttt acaaaaccat
29101 gtgaccaacc tggtagattt ttctgcaacg gcagagacct aaccattatc aacgtgacag
29161 caaatgacaa aggcttctat tatggaaccg actataaaag tagtttagat tataacatta
29221 ttgtactgcc atctaccact ccagcacccc gcacaactac tttctctagc agcagtgtcg
29281 ctaacaatac aatttccaat ccaacctttg ccgcgctttt aaaacgcact gtgaataatt
29341 ctacaacttc acatacaaca atttccactt caacaatcag catcatcgct gcagtgacaa
29401 ttggaatatc tattcttgtt tttaccataa cctactacgc ctgctgctat agaaaagaca
29461 aacataaagg tgatccatta cttagatttg atatttaatt tgttcttttt ttttatttac
29521 agtatggtga acaccaatca tggtacctag aaatttcttc ttcaccatac tcatctgtgc
29581 ttttaatgtt tgcgctactt tcacagcagt agccacagca accccagact gtataggagc
29641 atttgcttcc tatgcacttt ttgcttttgt tacttgcatc tgcgtatgta gcatagtctg
29701 cctggttatt aatttttttcc aacttctaga ctggatcctt gtgcgaattg cctacctgcg
29761 ccaccatccc gaataccgca accaaaatat cgcggcactt cttagactca tctaaaacca
29821 tgcaggctat actaccaata tttttgcttc tattgcttcc ctacgctgtc tcaaccccag
29881 ctgcctatag tactccacca gaacacctta gaaaatgcaa attccaacaa ccgtggtcat
29941 ttcttgcttg ctatcgagaa aaatcagaaa tccccccaaa tttaataatg attgctggaa
30001 taattaatat aatctgttgc accataattt catttttgat atacccccta tttgattttg
30061 gctggaatgc tcccaatgca catgatcatc cacaagaccc agaggaacac attccccccac
30121 aaaacatgca acatccaata gcgctaatag attacgaaag tgaaccacaa cccccactac
30181 tccctgctat tagttacttc aacctaaccg gcggagatga ctgaaacact caccacctcc
```

FIG. 2A-8

```
30241 aattccgccg aggatctgct cgatatggac ggccgcgtct cagaacaacg acttgcccaa
30301 ctacgcatcc gccagcagca ggaacgcgtg gccaaagagc tcagagatgt catccaaatt
30361 caccaatgca aaaaaggcat attctgtttg gtaaaacaag ccaagatatc ctacgagatc
30421 accgctactg accatcgcct ctcttacgaa cttggccccc aacgacaaaa atttacctgc
30481 atggtgggaa tcaaccccat agttatcacc caacaaagtg gagatactaa gggttgcatt
30541 cactgctcct gcgattccat cgagtgcacc tacaccctgc tgaagaccct atgcggccta
30601 agagacctgc taccaatgaa ttaaaaaaaa atgattaata aaaaatcact tacttgaaat
30661 cagcaataag gtctctgttg aaattttctc ccagcagcac ctcacttccc tcttcccaac
30721 tctggtattc taaaccccgt tcagcggcat actttctcca tactttaaag gggatgtcaa
30781 attttagctc ctctcctgta cccacaatct tcatgtcttt cttcccagat gaccaagaga
30841 gtccggctca gtgactcctt caaccctgtc taccctatg aagatgaaag cacctcccaa
30901 caccccttta taaacccagg gtttatttcc ccaaatggct tcacacaaag cccagacgga
30961 gttcttactt taaaatgttt aaccccacta acaaccacag gcggatctct acagctaaaa
31021 gtgggagggg gacttacagt ggatgacact gatggtacct tacaagaaaa catacgtgct
31081 acagcaccca ttactaaaaa taatcactct gtagaactat ccattggaaa tggattagaa
31141 actcaaaaca ataaactatg tgccaaattg ggaaatgggt taaaatttaa caacggtgac
31201 atttgtataa aggatagtat taacacctta tggactggaa taaaccctcc acctaactgt
31261 caaattgtgg aaaacactaa tacaaatgat ggcaaactta ctttagtatt agtaaaaaat
31321 ggagggcttg ttaatggcta cgtgtctcta gttggtgtat cagacactgt gaaccaaatg
31381 ttcacacaaa agacagcaaa catccaatta agattatatt ttgactcttc tggaaatcta
31441 ttaactgagg aatcagactt aaaaattcca cttaaaaata aatcttctac agcgaccagt
31501 gaaactgtag ccagcagcaa agcctttatg ccaagtacta cagcttatcc cttcaacacc
31561 actactaggg atagtgaaaa ctacattcat ggaatatgtt actacatgac tagttatgat
31621 agaagtctat ttcccttgaa catttctata atgctaaaca gccgtatgat ttcttccaat
31681 gttgcctatg ccatacaatt tgaatggaat ctaaatgcaa gtgaatctcc agaaagcaac
31741 atagctacgc tgaccacatc ccccttttc ttttcttaca ttacagaaga cgacaactaa
31801 aataaagttt aagtgttttt atttaaaatc acaaaattcg agtagttatt ttgcctccac
31861 cttcccattt gacagaatac accaatctct ccccacgcac agctttaaac atttggatac
31921 cattagagat agacattgtt ttagattcca cattccaaac agtttcagag cgagccaatc
31981 tggggtcagt gatagataaa aatccatcgc gatagtcttt taaagcgctt tcacagtcca
32041 actgctgcgg atgcgactcc ggagtttgga tcacggtcat ctggaagaag aacgatggga
32101 atcataatcc gaaaacggta tcggacgatt gtgtctcatc aaacccacaa gcagccgctg
32161 tctgcgtcgc tccgtgcgac tgctgtttat gggatcaggg tccacagttt cctgaagcat
32221 gattttaata gcccttaaca tcaactttct ggtgcgatgc gcgcagcaac gcattctgat
32281 ttcactcaaa tctttgcagt aggtacaaca cattattaca atattgttta ataaaccata
32341 attaaaagcg ctccagccaa aactcatatc tgatataatc gcccctgcat gaccatcata
32401 ccaaagttta atataaatta aatgacgttc cctcaaaaac acactaccca catacatgat
32461 ctcttttggc atgtgcatat taacaatctg tctgtaccat ggacaacgtt ggttaatcat
32521 gcaacccaat ataaccttcc ggaaccacac tgccaacacc gctcccccag ccatgcattg
32581 aagtgaaccc tgctgattac aatgacaatg aagaacccaa ttctctcgac cgtgaatcac
32641 ttgagaatga aaaatatcta tagtggcaca acatagacat aaatgcatgc atcttctcat
32701 aatttttaac tcctcaggat ttagaaacat atcccaggga ataggaagct cttgcagaac
32761 agtaaagctg gcagaacaag gaagaccacg aacacaactt acactatgca tagtcatagt
32821 atcacaatct ggcaacagcg ggtggtcttc agtcatagaa gctcgggttt cattttcctc
32881 acaacgtggt aactgggctc tggtgtaagg gtgatgtctg gcgcatgatg tcgagcgtgc
32941 gcgcaacctt gtcataatgg agttgcttcc tgacattctc gtattttgta tagcaaaacg
33001 cggccctggc agaacacact cttcttcgcc ttctatcctg ccgcttagcg tgttccgtgt
33061 gatagttcaa gtacagccac actcttaagt tggtcaaaag aatgctggct tcagttgtaa
33121 tcaaaactcc atcgcatcta attgttctga ggaaatcatc cacggtagca tatgcaaatc
33181 ccaaccaagc aatgcaactg gattgcgttt caagcaggag aggagaggga agagacggaa
33241 gaaccatgtt aatttttatt ccaaacgatc tcgcagtact tcaaattgta gatcgcgcag
33301 atggcatctc tcgcccccac tgtgttggtg aaaaagcaca gctaaatcaa aagaaatgcg
33361 attttcaagg tgctcaacgg tggcttccaa caaagcctcc acgcgcacat ccaagaacaa
33421 aagaatacca aaagaaggag cattttctaa ctcctcaatc atcatattac attcctgcac
33481 cattcccaga taattttcag ctttccagcc ttgaattatt cgtgtcagtt cttgtggtaa
33541 atccaatcca cacattacaa acaggtcccg gagggcgccc tccaccacca ttcttaaaca
33601 caccctcata atgacaaaat atcttgctcc tgtgtcacct gtagcgaatt gagaatggca
33661 acatcaattg acatgccctt ggctctaagt tcttctttaa gttctagttg taaaaactct
33721 ctcatattat caccaaactg cttagccaga agcccccggg gaacaagagc aggggacgct
33781 acagtgcagt acaagcgcag acctccccaa ttggctccag caaaaacaag attggaataa
33841 gcatattggg aaccaccagt aatatcatcg aagttgctgg aaatataatc aggcagagtt
33901 tcttgtagaa attgaataaa agaaaaattt gccaaaaaaa cattcaaaac ctctgggatg
33961 caaatgcaat aggttaccgc gctgcgctcc aacattgtta gttttgaatt agtctgcaaa
```

FIG. 2A-9

34021 aataaaaaaa aaacaagcgt catatcatag tagcctgacg aacaggtgga taaatcagtc
34081 tttccatcac aagacaagcc acagggtctc cagctcgacc ctcgtaaaac ctgtcatcgt
34141 gattaaacaa cagcaccgaa agttcctcgc ggtgaccagc atgaataagt cttgatgaag
34201 catacaatcc agacatgtta gcatcagtta aggagaaaaa acagccaaca tagcctttgg
34261 gtataattat gcttaatcgt aagtatagca aagccacccc tcgcggatac aaagtaaaag
34321 gcacaggaga ataaaaaata taattatttc tctgctgctg tttaggcaac gtcgcccccg
34381 gtccctctaa atacacatac aaagcctcat cagccatggc ttaccagaga aagtacagcg
34441 ggcacacaaa ccacaagctc taaagtcact ctccaacctc tccacaatat atatacacaa
34501 gccctaaact gacgtaatgg gactaaagtg taaaaaatcc cgccaaaccc aacacacacc
34561 ccgaaactgc gtcaccaggg aaaagtacag tttcacttcc gcaatcccaa caagcgtcac
34621 ttcctctttc tcacggtacg tcacatccca ttaacttaca acgtcatttt cccacggccg
34681 cgccgccccc tttaaccgtt aaccccacag ccaatcacca cacggcccac acttttaaa
34741 atcacctcat ttacatattg gcaccattcc atctataagg tatattattg atgatg
SEQ ID NO: 1

FIG. 2A-10

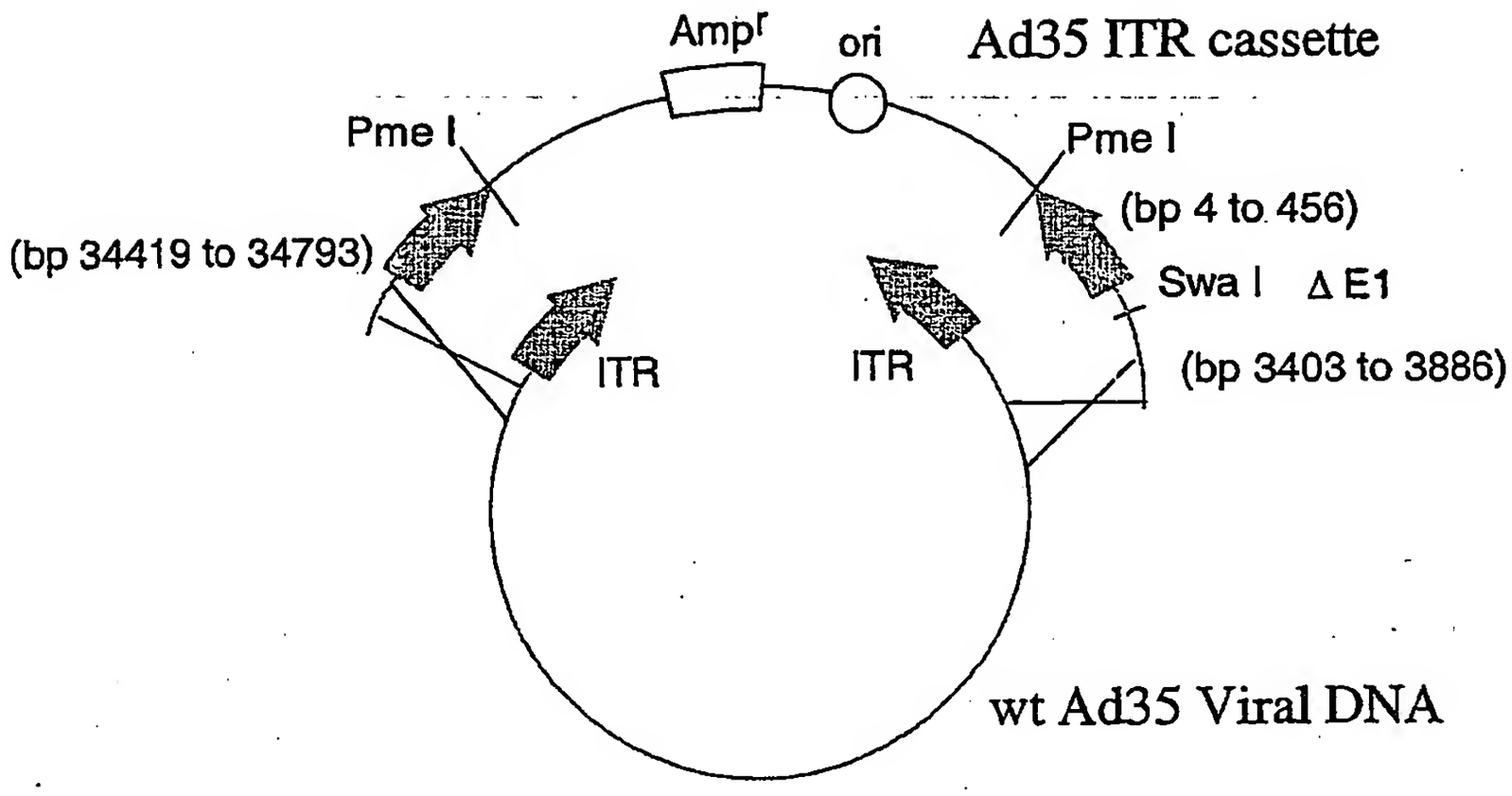

Ampr
ori
Ad35 ITR cassette
Pme I
(bp 34419 to 34793)
ITR
ITR
Pme I
(bp 4 to 456)
Swa I Δ E1
(bp 3403 to 3886)
wt Ad35 Viral DNA

FIG. 3

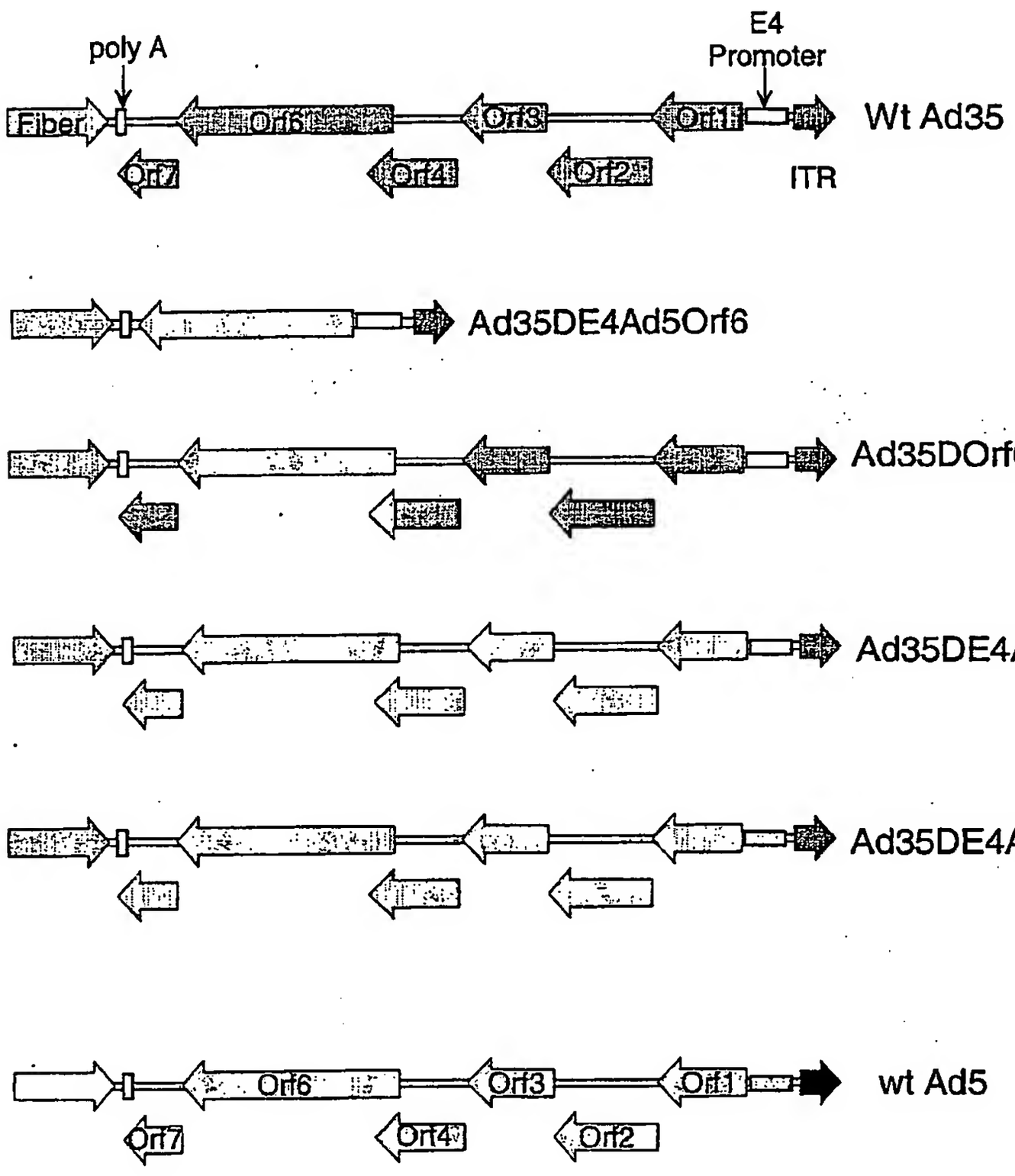

poly A
E4
Promoter
Fiber
Orf6
Orf3
Orf1
Orf7
Orf4
Orf2
ITR
Wt Ad35
Ad35DE4Ad5Orf6
Ad35DOrf6Ad5Orf6
Ad35DE4Ad5E4
Ad35DE4Ad5PE4
Orf6
Orf3
Orf1
Orf7
Orf4
Orf2
wt Ad5

FIG. 4

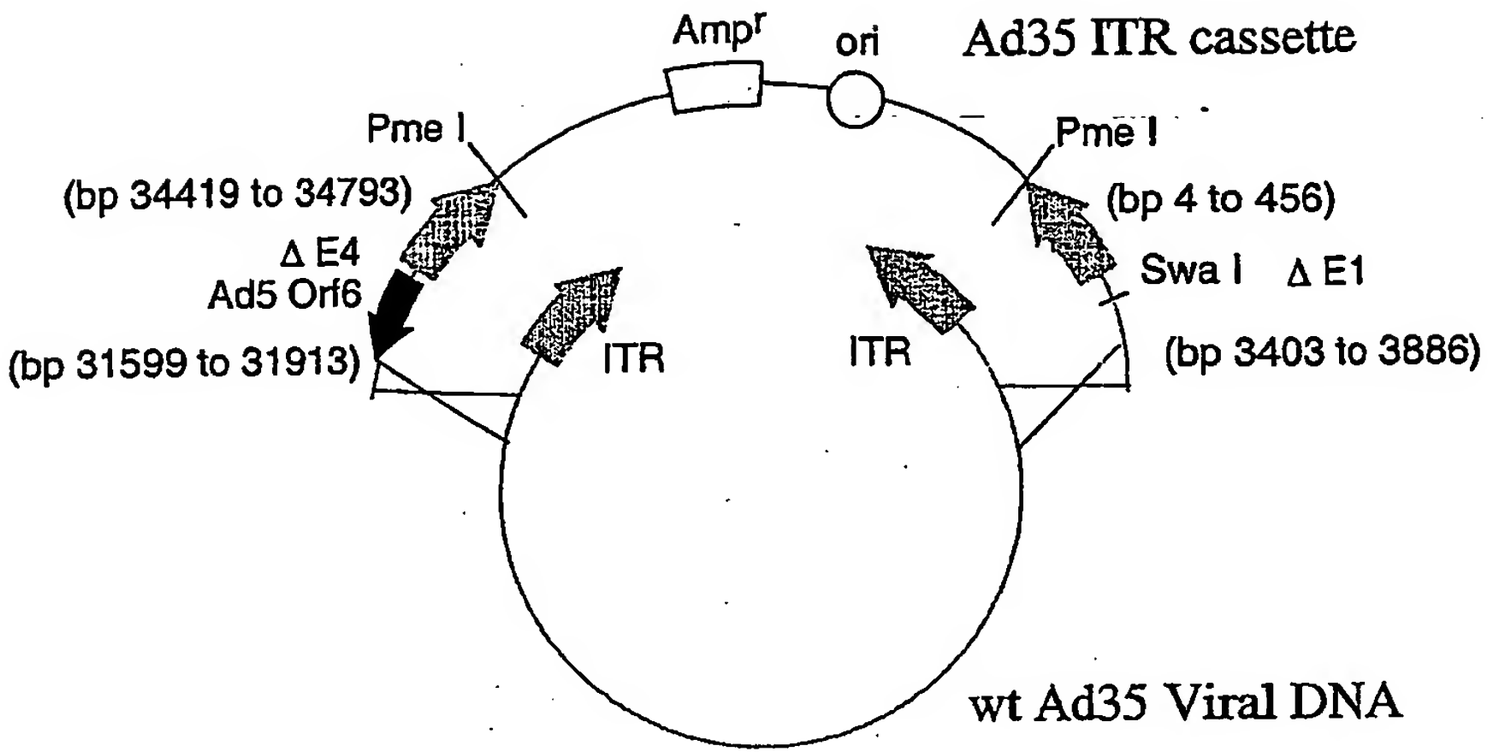

Amp^r
ori
Ad35 ITR cassette
Pme I
(bp 34419 to 34793)
Δ E4
Ad5 Orf6
(bp 31599 to 31913)
ITR
ITR
Pme I
(bp 4 to 456)
Swa I Δ E1
(bp 3403 to 3886)
wt Ad35 Viral DNA

FIG. 5

```
   1 ccattgcata cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca
  61 ttaccgccat gttgacattg attattgact agttattaat agtaatcaat tacggggtca
 121 ttagttcata gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct
 181 ggctgaccgc ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta
 241 acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac
 301 ttggcagtac atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt
 361 aaatggcccg cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag
 421 tacatctacg tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat
 481 gggcgtggat agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat
 541 gggagtttgt tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc
 601 ccattgacgc aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt
 661 ttagtgaacc gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga
 721 caccgggacc gatccagcct ccgcggccgg gaacggtgca ttggaacgcg gattccccgt
 781 gccaagagtg agatctaccA TGGGTGCTAG GGCTTCTGTG CTGTCTGGTG GTGAGCTGGA
 841 CAAGTGGGAG AAGATCAGGC TGAGGCCTGG TGGCAAGAAG AAGTACAAGC TAAAGCACAT
 901 TGTGTGGGCC TCCAGGGAGC TGGAGAGGTT TGCTGTGAAC CCTGGCCTGC TGGAGACCTC
 961 TGAGGGGTGC AGGCAGATCC TGGGCCAGCT CCAGCCCTCC CTGCAAACAG GCTCTGAGGA
1021 GCTGAGGTCC CTGTACAACA CAGTGGCTAC CCTGTACTGT GTGCACCAGA AGATTGATGT
1081 GAAGGACACC AAGGAGGCCC TGGAGAAGAT TGAGGAGGAG CAGAACAAGT CCAAGAAGAA
1141 GGCCCAGCAG GCTGCTGCTG GCACAGGCAA CTCCAGCCAG GTGTCCCAGA ACTACCCCAT
1201 TGTGCAGAAC CTCCAGGGCC AGATGGTGCA CCAGGCCATC TCCCCCCGGA CCCTGAATGC
1261 CTGGGTGAAG GTGGTGGAGG AGAAGGCCTT CTCCCCTGAG GTGATCCCCA TGTTCTCTGC
1321 CCTGTCTGAG GGTGCCACCC CCCAGGACCT GAACACCATG CTGAACACAG TGGGGGGCCA
1381 TCAGGCTGCC ATGCAGATGC TGAAGGAGAC CATCAATGAG GAGGCTGCTG AGTGGGACAG
1441 GCTGCATCCT GTGCACGCTG GCCCCATTGC CCCCGGCCAG ATGAGGGAGC CCAGGGGCTC
1501 TGACATTGCT GGCACCACCT CCACCCTCCA GGAGCAGATT GGCTGGATGA CCAACAACCC
1561 CCCCATCCCT GTGGGGGAAA TCTACAAGAG GTGGATCATC CTGGGCCTGA ACAAGATTGT
1621 GAGGATGTAC TCCCCCACCT CCATCCTGGA CATCAGGCAG GGCCCCAAGG AGCCCTTCAG
1681 GGACTATGTG GACAGGTTCT ACAAGACCCT GAGGGCTGAG CAGGCCTCCC AGGAGGTGAA
1741 GAACTGGATG ACAGAGACCC TGCTGGTGCA GAATGCCAAC CCTGACTGCA AGACCATCCT
1801 GAAGGCCCTG GGCCCTGCTG CCACCCTGGA GGAGATGATG ACAGCCTGCC AGGGGGTGGG
1861 GGGCCCTGGT CACAAGGCCA GGGTGCTGGC TGAGGCCATG TCCCAGGTGA CCAACTCCGC
1921 CACCATCATG ATGCAGAGGG GCAACTTCAG GAACCAGAGG AAGACAGTGA AGTGCTTCAA
1981 CTGTGGCAAG GTGGGCCACA TTGCCAAGAA CTGTAGGGCC CCCAGGAAGA AGGGCTGCTG
2041 GAAGTGTGGC AAGGAGGGCC ACCAGATGAA GGACTGCAAT GAGAGGCAGG CCAACTTCCT
2101 GGGCAAAATC TGGCCCTCCC ACAAGGGCAG GCCTGGCAAC TTCCTCCAGT CCAGGCCTGA
2161 GCCCACAGCC CCTCCCGAGG AGTCCTTCAG GTTTGGGGAG GAGAAGACCA CCCCCAGCCA
2221 GAAGCAGGAG CCCATTGACA AGGAGCTGTA CCCCCTGGCC TCCCTGAGGT CCCTGTTTGG
2281 CAACGACCCC TCCTCCCAGT AAaataaagc ccgggcagat ctgatctgct gtgccttcta
2341 gttgccagcc atctgttgtt tgcccctccc ccgtgccttc cttgaccctg gaaggtgcca
2401 ctcccactgt cctttcctaa taaaatgagg aaattgcatc gcattgtctg agtaggtgtc
2461 attctattct gggggggtggg gtggggcagc acagcaaggg ggaggattgg gaagacaata
2521 gcaggcatgc tggggatgcg gtgggctcta
SEQ ID NO: 2
```

FIG. 6

```
   1 ccattgcata cgttgtatcc atatcataat atgtacattt atattggctc atgtccaaca
  61 ttaccgccat gttgacattg attattgact agttattaat agtaatcaat tacggggtca
 121 ttagttcata gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct
 181 ggctgaccgc ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta
 241 acgccaatag ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac
 301 ttggcagtac atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt
 361 aaatggcccg cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag
 421 tacatctacg tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat
 481 gggcgtggat agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat
 541 gggagtttgt tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc
 601 ccattgacgc aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctcgt
 661 ttagtgaacc gtcagatcgc ctggagacgc catccacgct gttttgacct ccatagaaga
 721 caccgggacc gatccagcct ccgcggccgg gaacggtgca ttggaacgcg gattccccgt
 781 gccaagagtg agatcgatct aagtaagctt CCTGCATGCT GCTGCTGCTG CTGCTGCTGG
 841 GCCTGAGGCT ACAGCTCTCC CTGGGCATCA TCCCAGTTGA GGAGGAGAAC CCGGACTTCT
 901 GGAACCGCGA GGCAGCCGAG GCCCTGGGTG CCGCCAAGAA GCTGCAGCCT GCACAGACAG
 961 CCGCCAAGAA CCTCATCATC TTCCTGGGCG ATGGGATGGG GGTGTCTACG GTGACAGCTG
1021 CCAGGATCCT AAAAGGGCAG AAGAAGGACA AACTGGGGCC TGAGATACCC CTGGCCATGG
1081 ACCGCTTCCC ATATGTGGCT CTGTCCAAGA CATACAATGT AGACAAACAT GTGCCAGACA
1141 GTGGAGCCAC AGCCACGGCC TACCTGTGCG GGGTCAAGGG CAACTTCCAG ACCATTGGCT
1201 TGAGTGCAGC CGCCCGCTTT AACCAGTGCA ACACGACACG CGGCAACGAG GTCATCTCCG
1261 TGATGAATCG GGCCAAGAAA GCAGGGAAGT CAGTGGGAGT GGTAACCACC ACACGAGTGC
1321 AGCACGCCTC GCCAGCCGGC ACCTACGCCC ACACGGTGAA CCGCAACTGG TACTCGGACG
1381 CCGACGTGCC TGCCTCCGCC CGCCAGGAGG GGTGCCAGGA CATCGCTACG CAGCTCATCT
1441 CCAACATGGA CATTGACGTG ATCCTAGGTG GAGGCCGAAA GTACATGTTT CGCATGGGAA
1501 CCCCAGACCC TGAGTACCCA GATGACTACA GCCAAGGTGG GACCAGGCTG GACGGGAAGA
1561 ATCTGGTGCA GGAATGGCTG GCGAAGCGCC AGGGTGCCCG GTATGTGTGG AACCGCACTG
1621 AGCTCATGCA GGCTTCCCTG GACCCGTCTG TGACCCATCT CATGGGTCTC TTTGAGCCTG
1681 GAGACATGAA ATACGAGATC CACCGAGACT CCACACTGGA CCCCTCCCTG ATGGAGATGA
1741 CAGAGGCTGC CCTGCGCCTG CTGAGCAGGA ACCCCCGCGG CTTCTTCCTC TTCGTGGAGG
1801 GTGGTCGCAT CGACCATGGT CATCATGAAA GCAGGGCTTA CCGGGCACTG ACTGAGACGA
1861 TCATGTTCGA CGACGCCATT GAGAGGGCGG GCCAGCTCAC CAGCGAGGAG GACACGCTGA
1921 GCCTCGTCAC TGCCGACCAC TCCCACGTCT TCTCCTTCGG AGGCTACCCC CTGCGAGGGA
1981 GCTCCATCTT CGGGCTGGCC CCTGGCAAGG CCCGGGACAG GAAGGCCTAC ACGGTCCTCC
2041 TATACGGAAA CGGTCCAGGC TATGTGCTCA AGGACGGCGC CCGGCCGGAT GTTACCGAGA
2101 GCGAGAGCGG GAGCCCCGAG TATCGGCAGC AGTCAGCAGT GCCCCTGGAC GAAGAGACCC
2161 ACGCAGGCGA GGACGTGGCG GTGTTCGCGC GCGGCCCGCA GGCGCACCTG GTTCACGGCG
2221 TGCAGGAGCA GACCTTCATA GCGCACGTCA TGGCCTTCGC CGCCTGCCTG GAGCCCTACA
2281 CCGCCTGCGA CCTGGCGCCC CCCGCCGGCA CCACCGACGC CGCGCACCCG GGTTAAcccg
2341 tggtccccgc gttgcttcct ctgctggccg ggacatcagg tggcccccgc tgaattggaa
2401 tcgatcagaa ttgatctgat ctgctgtgcc ttctagttgc cagccatctg ttgtttgccc
2461 ctccccccgtg ccttccttga ccctggaagg tgccactccc actgtccttt cctaataaaa
2521 tgaggaaatt gcatcgcatt gtctgagtag gtgtcattct attctggggg gtggggtggg
2581 gcagcacagc aaggggggagg attgggaaga caatagcagg catgctgggg atgcggtggg
2641 ctcta
```

SEQ ID NO: 3

FIG. 7

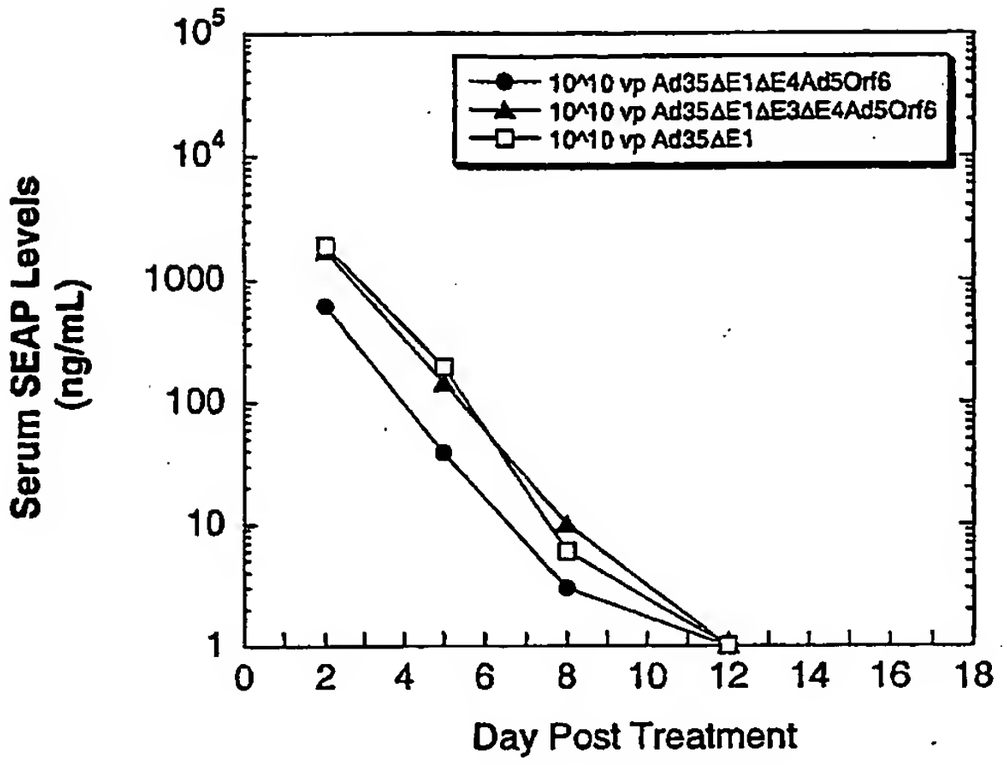

10^10 vp Ad35ΔE1ΔE4Ad5Orf6
10^10 vp Ad35ΔE1ΔE3ΔE4Ad5Orf6
10^10 vp Ad35ΔE1
Serum SEAP Levels (ng/mL)
10^5
10^4
1000
100
10
1
0
2
4
6
8
10
12
14
16
18
Day Post Treatment

FIG. 8

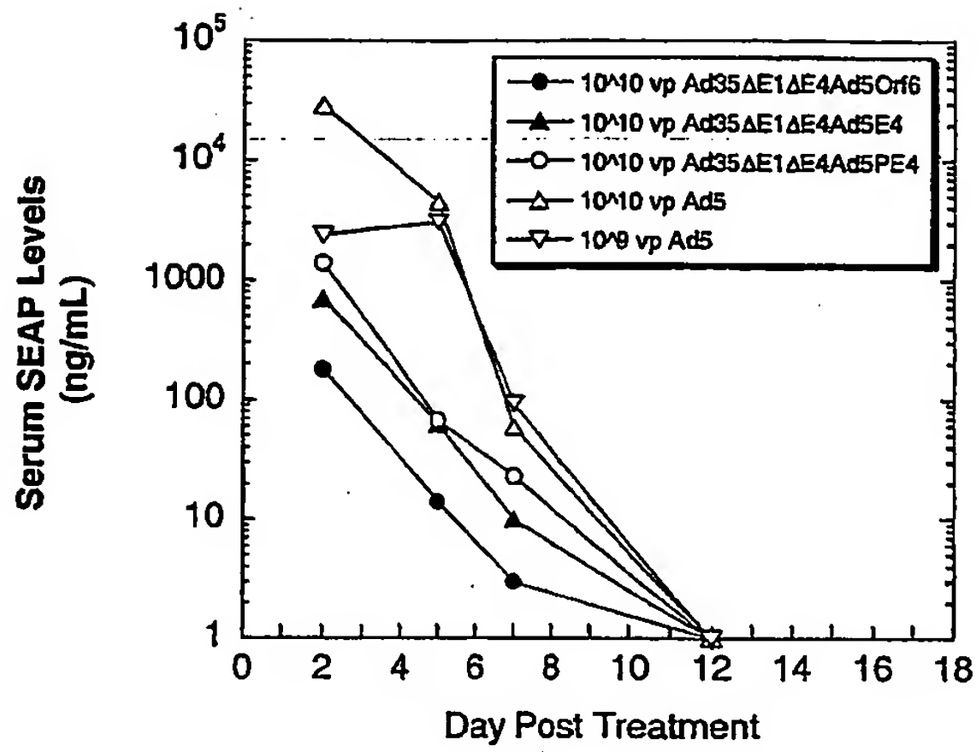

Serum SEAP Levels (ng/mL)
10^5
10^4
1000
100
10
1
0 2 4 6 8 10 12 14 16 18
Day Post Treatment
10^10 vp Ad35ΔE1ΔE4Ad5Orf6
10^10 vp Ad35ΔE1ΔE4Ad5E4
10^10 vp Ad35ΔE1ΔE4Ad5PE4
10^10 vp Ad5
10^9 vp Ad5

FIG. 9

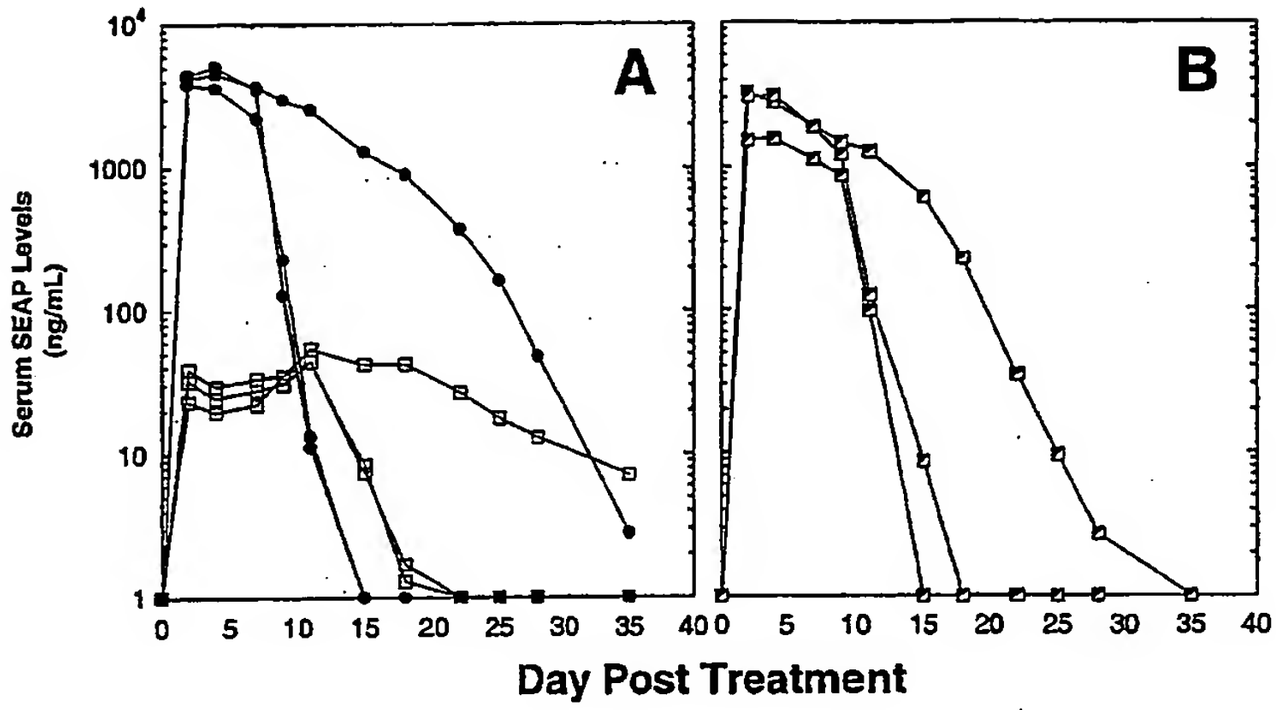

A
B
Serum SEAP Levels (ng/mL)
10^4
1000
100
10
1
0 5 10 15 20 25 30 35 40
0 5 10 15 20 25 30 35 40
Day Post Treatment

FIG. 10A-B

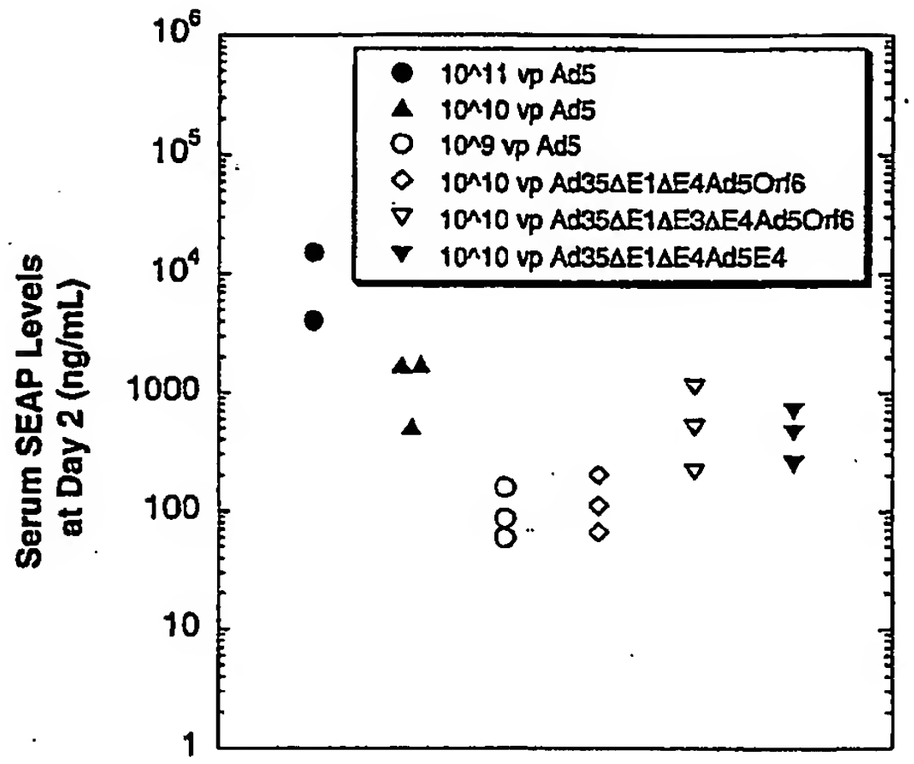

Serum SEAP Levels at Day 2 (ng/mL)
10^6
10^5
10^4
1000
100
10
1
10^11 vp Ad5
10^10 vp Ad5
10^9 vp Ad5
10^10 vp Ad35ΔE1ΔE4Ad5Orf6
10^10 vp Ad35ΔE1ΔE3ΔE4Ad5Orf6
10^10 vp Ad35ΔE1ΔE4Ad5E4

FIG. 11

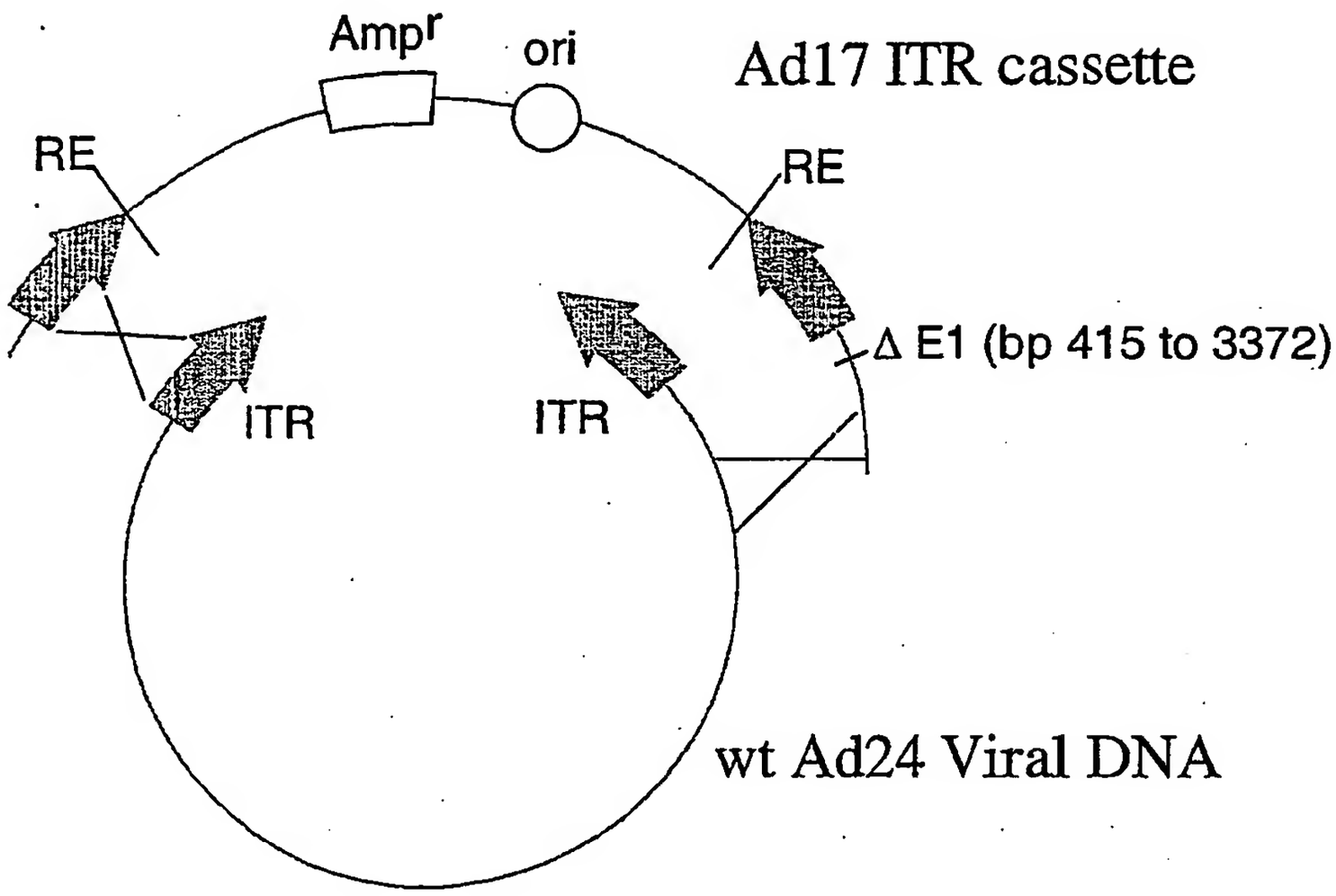

Ampr
ori
Ad17 ITR cassette
RE
RE
Δ E1 (bp 415 to 3372)
ITR
ITR
wt Ad24 Viral DNA

FIG. 12

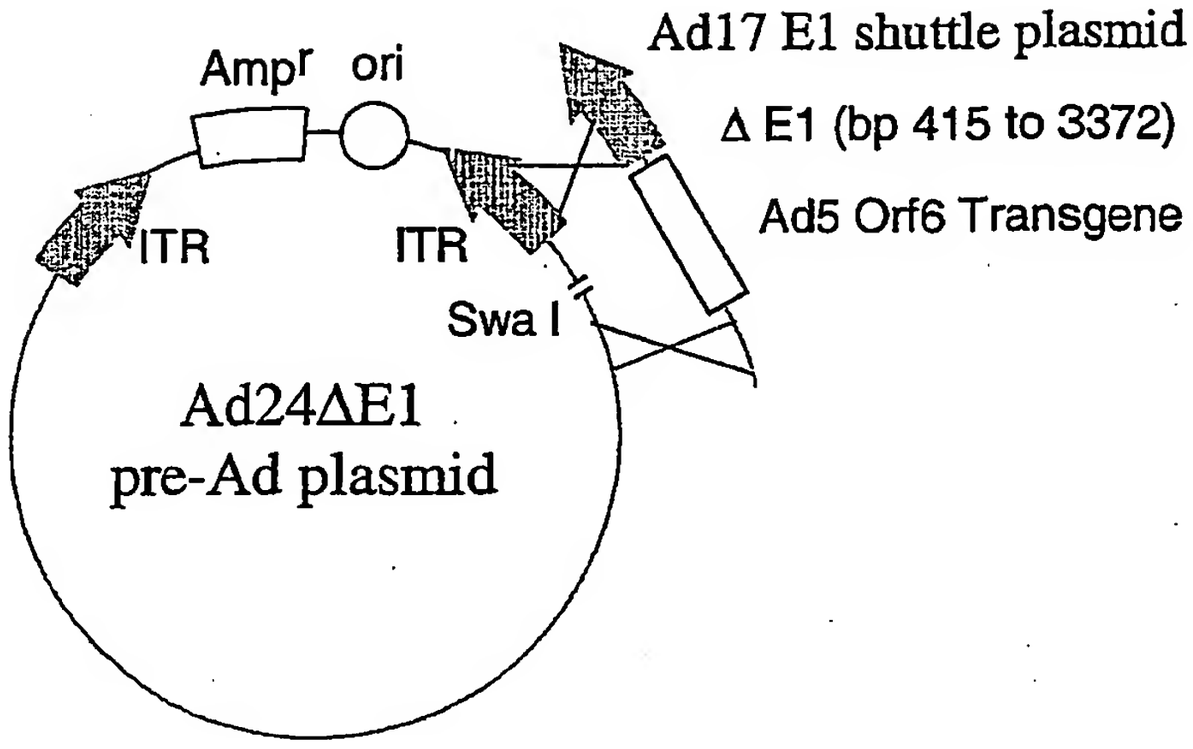

Ampr ori
ITR
ITR
Swa I
Ad24ΔE1
pre-Ad plasmid
Ad17 E1 shuttle plasmid
Δ E1 (bp 415 to 3372)
Ad5 Orf6 Transgene

FIG. 13

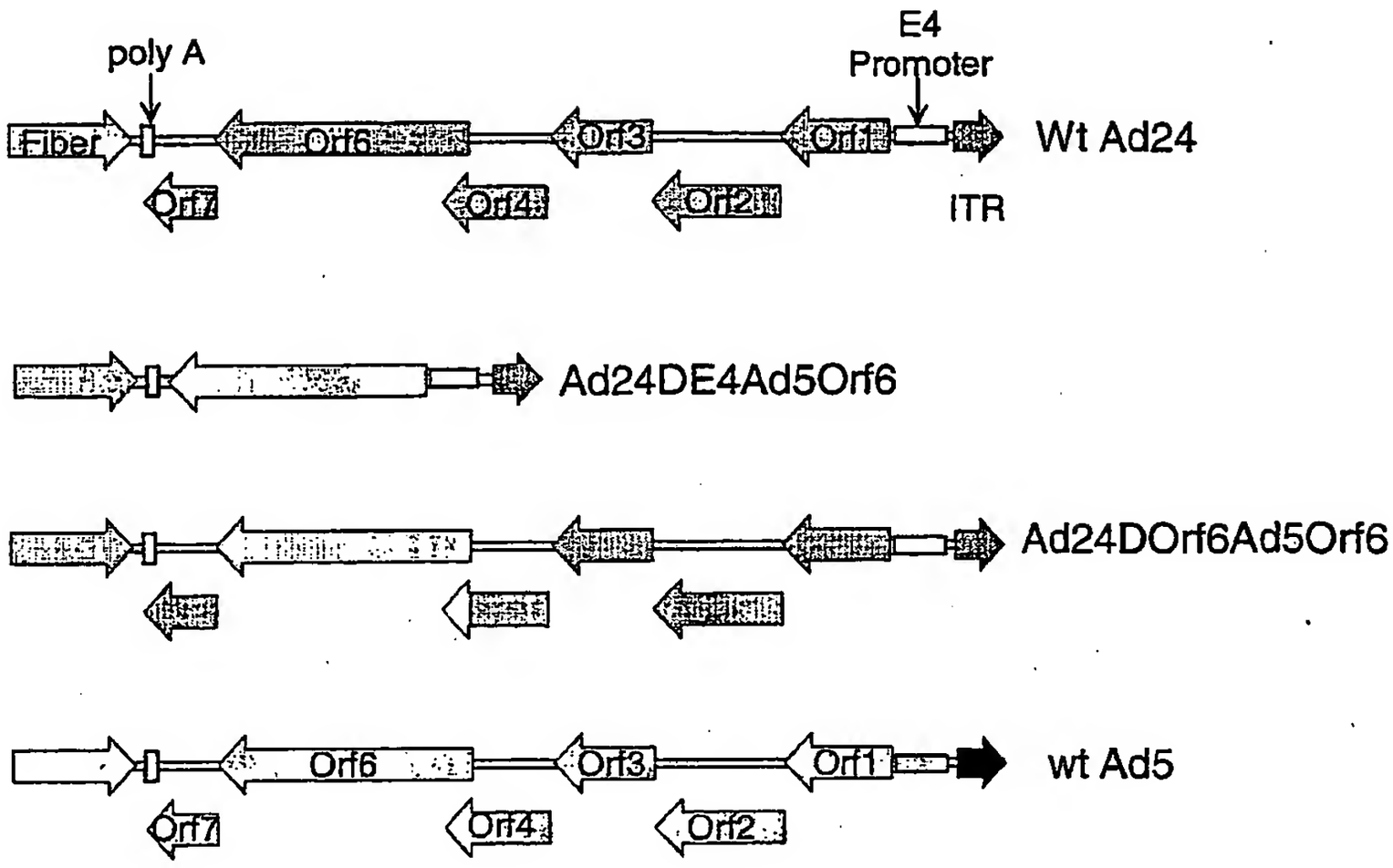

poly A
E4
Promoter
Fiber
Orf6
Orf3
Orf1
Wt Ad24
Orf7
Orf4
Orf2
ITR
Ad24DE4Ad5Orf6
Ad24DOrf6Ad5Orf6
Orf6
Orf3
Orf1
wt Ad5
Orf7
Orf4
Orf2

FIG. 14

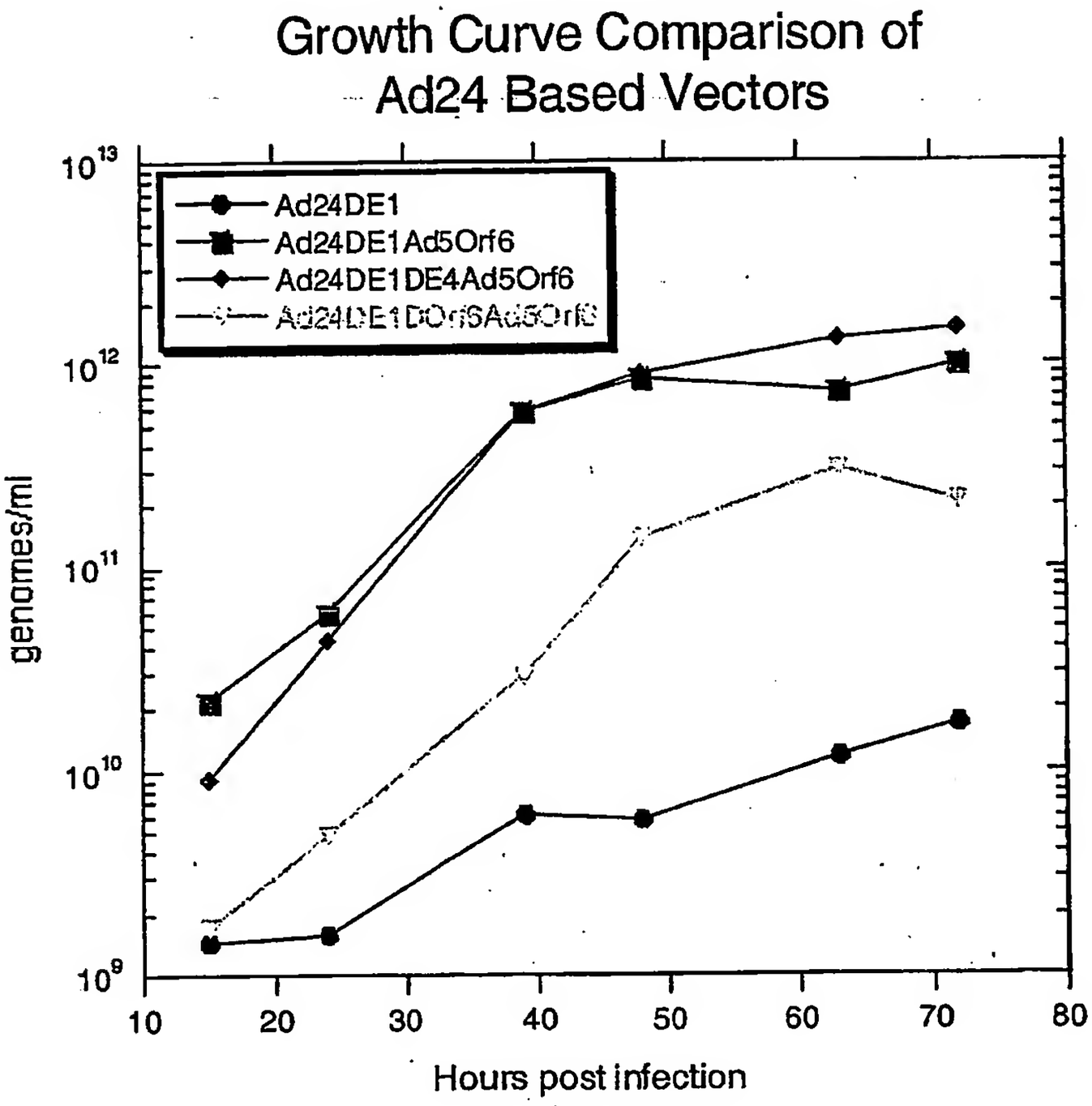

Growth Curve Comparison of
Ad24 Based Vectors
Ad24DE1
Ad24DE1Ad5Orf6
Ad24DE1DE4Ad5Orf6
Ad24DE1DOrfSAd5Orf6
genomes/ml
10^{13}
10^{12}
10^{11}
10^{10}
10^{9}
10
20
30
40
50
60
70
80
Hours post infection

FIG. 15

```
   1 catcatcaat aatatacccc acaaagtaaa caaaagttaa catgcaaatg agcttttgaa
  61 tttagggcgg ggccagcgct gattggacga gagaagatga tgcaaatgac gtcacgacgc
 121 acggctaacg gtcgccgcgg aggcgtggcc tagcccggaa gcaagtcgcg gggctgatga
 181 cgtataaaaa agcggacttt agacccggaa acggccgatt ttcccgcggc cacgcccgga
 241 tatgaggtaa ttctgggcgg atgcaagtaa aattaggtca ttttggcgcg aaaactgaat
 301 gaggaagtga aaagtgaaaa ataccggtcc cgcccagggc ggaatattta ccgagggccg
 361 agagactttg accgattacg tgggggtttc gattgcggtg ttttttcgcg aatttccgcg
 421 tccgtgtcaa agtccggtgt ttatgtcaca gatcagctga tccacagggt atttaaacca
 481 gtcgagcccg tcaagaggcc actcttgagt gccagcgagt agagatttct ctgagctccg
 541 ctcccagagt ctgagaaaaa tgagacacct gcgcctcctt tcttcaactg tgcctattga
 601 catggccgca ttattgctgg aggattatgt gagtacaata ttggaggacg aactgcatcc
 661 atctccattt gagctgggac ctacacttca ggacctatat gatttggagg tagatgccca
 721 tgatgacgac ccgaacgaag aggctgtgaa tttaatattt ccagaatctc tgattcttca
 781 ggctgacata gccagcgaag ctgtacctac accacttcat acaccgactc tgtcacccat
 841 acctgaattg gaagaggagg acgagctaga cctccgatgt tatgaggaag gttttcctcc
 901 cagcgattca gaggacgaac agggtgagca gagcatggct ctaatctcaa aatatgcttg
 961 tgtggttgtg gaagagcatt ttgtgttgga caatcctgag gtgcccgggc aaggctgtag
1021 atcctgccag taccaccggg ataagaccgg agacacgaac gcctcctgcg ctctgtgtta
1081 catgaaaaag aacttcagct ttatttacag taagtggagt gaatgtgaga gagactgagt
1141 gcttaacaca taactgggta atgcttaaac agctgtgcta agtgtggttt atttttgttt
1201 ctaggtccgg tgtcagagga tgagtcatca ccctcagaag aagaccaccc gtgtcccccc
1261 gagctgtcag gcgaaacgcc cctgcaagtg cacagaccca ccccagtcag acccagtggc
1321 gagaggcgag cagctgttga aaaaattgag gacttgttac atgacatggg tggggatgaa
1381 cctttggacc tgagcttgaa acgccccagg aactaggctc agctgtgctt agtcatgtgt
1441 aaataaagtt gtacaataaa agtatatgtg acgcatgcaa ggtgtggttt atgactcatg
1501 ggcgtggctt agtcctatat aagtggcaac acctgggcac tggggcacag accttcaggg
1561 agttcctgat ggatgtgtgg actatccttg cagactttag caagacacgc cggcttgtag
1621 aggatagttc agacgggtgc tccgggttct ggagacactg gtttggaact cctctatctc
1681 gtctggtgta cacagttaag aaggattata acgaggaatt tgaaaatctt tttgctgatt
1741 gctctggcct gctagattct ctaaatctcg gccaccagtc ccttttccag gaaagggtac
1801 tccacagcct tgatttttca agcccagggc gcactacagc cggggttgct tttgtggttt
1861 ttctggttga caaatggagc cagaacaccc aactgagcag gggctacatt ctggacttcg
1921 cagccatgca cctgtggagg gcatgggtga ggcagcgggg acagagaatc ttgaactact
1981 ggcttataca gccagcagct ccgggtcttc ttcgtctaca cagacaaaca tccatgttgg
2041 aggaagaaat gaggcaggcc atggacgaga acccgaggag cggcctggac cctccgtcgg
2101 aagaggagct ggattgaatc aggtatccag cctgtaccca gagcttagca gggtgctgac
2161 atccatggcc aggggagtga agagggagag gagcgatggg ggcaataccg ggatgatgac
2221 cgagctgacg gccagcctga tgaatcgcaa gcgtccagag cgcattacct ggcacgagct
2281 acagatggag tgtagggatg aggtgggcct gatgcaggat aaatatggcc tggagcagat
2341 aaaaacccac tggttgaacc cagatgagga ttgggaggag gccattaaga aatatgccaa
2401 gatagccctg cgcccagatt gcaagtacag ggtgaccaag acggtgaata tcagacatgc
2461 ctgctacatc tcggggaacg gggcagaggt ggtcatcgat accctggaca aggccgcctt
2521 caggtgttgc atgatgggaa tgagagccgg agtgatgaat atgaattcca tgattttcat
2581 gaacatgaag ttcaatggag agaagtttaa tggggtgatg ttcatggcca acagtcacat
2641 gaccctgcac ggctgcagtt tcttcggctt caacaatatg tgcgcagagg tctggggcgc
2701 tgctaagatc aggggatgta agttttatgg ctgctggatg ggcgtggtcg gaagacccaa
2761 gagcgagatg tctgtgaagc agtgtgtgtt tgagaaatgc tacctgggag tctctaccga
2821 gggcaatgct agagtgagac attgctcttc cctggagacg ggctgcttct gcctggtgaa
2881 gggcacagcc tctctgaagc ataatatggt gaagggctgc acggatgagc gcatgtacaa
2941 catgctgaca tgcgactcgg gggtctgcca tatcctgaag aacatccatg tgacctccca
3001 cccccggaag aagtggccag tgtttgagaa taacctactg atcaagtgcc acatgcacct
3061 gggcgccaga aggggcacct tccagccgta ccagtgcaac tttagccaga ccaagctgct
3121 gctggagaac gatgccttct ccagggtgaa cctgaacggc atctttgaca tggatgtctc
3181 ggtgtacaag atcctgagat acgatgagac caagtccagg gtgcgcgctt gcgagtgcgg
3241 gggcagacac accaggatgc aaccagtggc cctggatgtg accgaggagc tgaggcccga
3301 ccacctggtg atggcttgta ccgggaccga gttcagctcc agtggggagg acacagatta
3361 gaggtaggtt gagtattagt gggcgtggct aaggtgacta taaaggcggg tgtcttacga
3421 gggtcttttt gcttttctgc agacatcatg aacgggactg gcggggcctt cgaaggggga
3481 ctttttagcc cttatttgac aacccgcctg ccgggatggg ccggagttcg tcagaatgtg
3541 atgggatcga cggtggacgg gcgtccagtg cttccagcaa attcctcgac catgacctac
3601 gcgaccgtgg ggaactcgtc gctcgacagc accgccgcag ccgcggcagc cgcagccgcc
```

FIG. 16A-1

```
3661 atgacagcga cgagactggc ttcgagctac atgcccagca gcagcagtag cccctctgtg
3721 cccagttcca tcatcgccga ggagaaactg ctggccctgc tggccgagct ggaagccctg
3781 agccgccagc tggccgccct gacccagcag gtgtccgagc tccgcgaaca gcagcagcag
3841 caaaataaat gattcaataa acacagattc tgattcaaac agcaaagcat ctttattatt
3901 tatttttcg cgcgcggtag gccctggtcc acctctcccg atcattgaga gtgcggtgga
3961 ttttttccag gacccggtag aggtgggatt ggatgttgag gtacatgggc atgagcccgt
4021 cccgggggtg gaggtagcac cactgcatgg cctcgtgctc tggggtcgtg ttgtagatga
4081 tccagtcata gcaggggcgc tgggcgtggt gctggatgat gtccttgagg aggagactga
4141 tggccacggg gagccccttg gtgtaggtgt tggcgaagcg gttgagctgg gagggatgca
4201 tgcgggggga gatgatgtgg agtttggcct ggatcttgag gttggcgatg ttgccaccca
4261 gatcccgcct ggggttcatg ttgtgcagga ccaccagaac ggtgtagccc gtgcacttgg
4321 ggaacttgtc atgcaacttg gaagggaatg cgtgaaagaa tttggagacg cccttgtgcc
4381 cacccaggtt ttccatgcac tcatccatga tgatggcgat gggcccgtgg gctgcggctt
4441 tggcaaagac gtttctgggg tcagagacat cgtaattatg ctcctgggtg agatcatcat
4501 aagacatttt aatgaatttg gggcggaggg tgccagattg ggggacaatg gttccctcgg
4561 gccccggggc gaagttcccc tcacatattt gcatctccca ggctttcatc tcggaggggg
4621 ggatcatgtc cacctgcggg gcgatgaaaa aaacggtttc cggggcgggg gtgatgagct
4681 gcgaggagag caggtttctc aacagctggg acttgccgca cccggtcggg ccgtagatga
4741 ccccgatgac gggttgcagg tggtagttca aggacatgca gctgccgtcg tcccgggagga
4801 gggggggccac ctcgttgagc atgtctctga cttggaggtt ttcccggacg agctcgccga
4861 ggaggcggtc cccgcccagc gagagcagct cttgcaggga agcaaagttt ttcaggggct
4921 tgagcccgtc ggccatgggc atcttggcga gggtctgcga gaggagttcg aggcggtccc
4981 agagctcggt gacgtgctct acggcatctc gatccagcag acttcctcgt ttcgggggtt
5041 gggacgactg cgactgtagg gcacgagacg atgggcgtcc agcgctgcca gcgtcatgtc
5101 cttccagggt ctcagtgtcc gcgtgagcgt ggtctccgtc acggtgaagg ggtgggcccc
5161 gggctgtgcg cttgcaaggg tgcgcttgag actcatcctg ctggtgctga aacgggcacg
5221 gtcttcgccc tgcgcgtcgg cgagatagca gttgaccatg agctcgtagt tgagggcctc
5281 ggcggcgtgg cccttggcgc ggagcttgcc cttggaagag cgcccgcagg cgggacagag
5341 gagggattgc agggcgtaga gcttgggtgc gagaaagacg gactcggggg cgaaagcatc
5401 cgctccgcag tgggcgcaga cggtctcgca ctcgaccagc caggtgagct cgggctgctc
5461 ggggtcaaaa accagttttc ccccgttctt tttgatgcgc ttcttacctc gcgtctccat
5521 gagtctgtgt ccgcgctcgg tgacaaacag gctgtctgtg tccccgtaga cggacttgat
5581 gggcctgtcc tgcaggggcg tcccgcggtc ctcctcgtag agaaactcgg accactctga
5641 gacgaaggcg cgcgtccacg ccaagacaaa ggaggccacg tgcgaggggt agcggtcgtt
5701 gtccaccagg gggtccacct tttccacggt atgcagacac atgtcccccc cctccgcatc
5761 caagaaggtg attggcttgt aggtgtaggc cacgtgaccc ggggtccccg acgggggggt
5821 ataaaagggg gcgggtctgt gctcgtcctc actctcttcc gcgtcgctgt ccacgagcgc
5881 cagctgttgg ggtaggtatt ccctttcgag agcgggcatg acctcggcac tcaggttgtc
5941 agtttctaga aacgaggagg atttgatgtt ggcttgccct gccgcaatgc tttttaggag
6001 actttcatcc atctggtcag aaaagactat ttttttattg tcaagcttgg tggcgaagga
6061 gccatagagg gcgttggaga gaagcttggc gatggatctc atggtctgat ttttgtcacg
6121 gtcggctcgc tccttggccg cgatgttgag ctggacatac tcgcgcgcga cgcacttcca
6181 ttcggggaag acggtggtgc gctcgtcggg cacgatcctg acgcgccagc cgcggttatg
6241 cagggtgacc agatccacgc tggtggccac ctcgccgcgc aggggctcgt tggtccagca
6301 gaggcgtccg cccttgcgcg agcagaacgg gggcagcaca tcaagcagat gctcgtcagg
6361 ggggtccgca tcgatggtga agatgcccgg acagagttcc ttgtcaaaat aatcgatttt
6421 tgaggatgca tcatccaagg ccatctgcca ctcgcgggcg gccagcgctc gctcgtaggg
6481 gttgaggggc ggaccccagg gcatgggatg cgtcagggcg gaggcgtaca tgccgcagat
6541 gtcgtagaca tagatgggct ccgagaggat gccgatgtag gtgggataac agcgcccccc
6601 gcggatgctg gcgcgcacgt agtcatacaa ctcgtgcgag ggggccaaga aggcggggcc
6661 gagattggtg cgctggggct gctcggcgcg gaagacgatc tggcgaaaga tggcatgcga
6721 gttggaggag atggtgggcc gttggaagat gttaaagtgg gcatgaggca gacgaaccga
6781 gtcgcggatg aagtgcgcgt aggagtcttg cagcttggcg acgagctcgg cggtgacgag
6841 gacgtccatg gcgcagtagt ccagcgtttc gcggatgatg tcataacccg cctctccttt
6901 cttctcccat agctcgcggt tgagggcgta ctcctcgtca tccttccagt actcccggag
6961 cgggaatcct cgatcgtccg cacggtaaga gcccagcatg tagaaatggt tcacggcctt
7021 gtagggacag cagcccttct ccacggggag ggcgtaagct tgagcggcct tgcggagcga
7081 ggtgtgcgtc agggcgaagg tatccctgac catgactttc aagaactggt acttgaaatc
7141 cgagtcgtcg cagccgccgt gctcccagag ctcgaaatcg gtgcgcttct tcgagagggg
7201 gttaggcaga gcgaaagtga cgtcattgaa gagaatcttg cctgcccgcg gcatgaaatt
7261 gcgggtgatg cggaaagggc ccgggacgga ggctcggttg ttgatgacct gggcggcgag
```

FIG. 16A-2

```
7321 gacgatctcg tcgaagccgt tgatgttgtg cccgacgatg tagagttcca tgaatcgcgg
7381 gcggccttta atgtgcggca gcttttgag ctcctcgtag gtgaggtcct cggggcaatg
7441 cagtccgtgc tgctcgagcg cccactcctg gagatgtggg ttggcttgca tgaatgaagc
7501 ccagagctcg cgggccataa gggtctggag ctcgtcgcga aagaggcgga actgctggcc
7561 cacggccatc ttttctgggg tgacgcagta gaaagtaagg gggtcccgct cccagcgatc
7621 ccagcgtaag cgcacggcta gatcgcgagc gagggcgacc agctctgggt cccccgagaa
7681 tttcataacc agcataaagg ggacgagctg cttgccgaag gaccccatcc aggtgtaggt
7741 ttctacatcg taggtgacaa agagccgctc cgtgcgagga tgagagccga ttgggaagaa
7801 ctggatttcc tgccaccagt tggacgagtg gctgttgatg tgatgaaagt agaaatcccg
7861 ccggcgaacc gagcactcgt gctgatgctt gtaaaagcgt ccgcagtact cgcagcgctg
7921 cacgggctgt acctcatcca cgagatacac agcgcgtccc ttgaggagga acttcaggag
7981 tggcggccct ggctggtggt tttcatgttc gcctgcgtgg gactcaccct ggggctcctc
8041 gaggacggag aggctgacga gcccgcgcgg gagccaggtc cagatctcgg cgcggcgggg
8101 gcggagagcg aagacgaggg cgcgcagttg ggagctgtcc atggtgtcgc ggagatccag
8161 gtccgggggc agggttctga ggttgacctc gtagaggcgg gtgagggcgt gcttgagatg
8221 cagatggtac ttgatctcca cgggtgagtt ggtggctgtg tccacgcatt gcatgagccc
8281 gtagctgcgc ggggccacga ccgtgccgcg gtgcgctttt agaagcggtg tcgcggacgc
8341 gctcccggcg gcagcggcgg ttccggcccc gcgggcaggg gcggcagagg cacgtcggcg
8401 tggcgctcgg gcaggtcccg gtgctgcgcc ctgagagcgc tggcgtgcgc gacgacgcgg
8461 cggttgacat cctggatctg ccgcctctgc gtgaagacca ccggccccgt gactttgaac
8521 ctgaaagaca gttcaacaga atcaatctcg gcgtcattga cggcggcctg acgcaggatc
8581 tcttgcacgt cgcccgagtt gtcctggtag gcgatctcgg acatgaactg ctcgatctcc
8641 tcctcctgga gatcgccgcg gcccgcgcgc tccacggtgg cggcgaggtc attggagatg
8701 cgacccatga gctgcgagaa ggcgcccagg ccgctctcat tccagacgcg gctgtagacc
8761 acgtccccgt cggcgtcgcg cgcgcgcatg accacctgcg cgaggttgag ctccacgtgc
8821 cgcgtgaaga cggcgtagtt gcgcaggcgc tggaagaggt agtttagggt ggtggcgatg
8881 tgctcggtga cgaagaagta catgatccag cggcgcaggg gcatctcgct gatgtcgccg
8941 atggcctcca gcctttccat ggcctcgtag aaatccacag cgaagttgaa aaactgggcg
9001 ttgcgggccg agaccgtgag ctcgtcctcc aggagcctga tgagttcggc gatggtggcg
9061 cgcacctcgc gctcgaaatc cccgggggcc tcctcctctt cctcttcttc catgacgacc
9121 tcttcttcta tttcttcctc tgggggcggt ggtggtggcg gggcccgacg acgacggcga
9181 cgcaccggga gacggtcgac gaagcgctcg atcatctccc cgcggcggcg acgcatggtt
9241 tcggtgacgg cgcgaccccg ttcgcgagga cgcagcgtga agacgccgcc ggtcatctcc
9301 cggtaatggg gcgggtcccc gttgggcagc gagagggcgc tgacgatgca tcttatcaat
9361 tgcggtgtag gggacgtgag cgcgtcgaga tcgaccggat cggagaatct ttcgaggaaa
9421 gcgtctagcc aatcgcagtc gcaaggtaag ctcaaacacg tagcagccct gtggacgctg
9481 ttagaattgc ggttgctgat gatgtaattg aagtaggcgt ttttaaggcg gcggatggtg
9541 gcgaggagga ccaggtcctt gggtcccgct tgctggatgc gaagccgctc ggccatgccc
9601 caggcctggc cctgacaccg gctcaggttc ttgtagtagt catgcatgag cctctcaatg
9661 tcatcactgg cggaggcgga gtcttccatg cgggtgaccc cgacgccgct gagcggctgc
9721 acgagcgcca ggtcggcgac gacgcgctcg gcgaggatgg cctgttgcac gcgggtgagg
9781 gtgtcctgga agtcgtccat gtcgacgaag cggtggtagg ccccggtgtt gatggtgtag
9841 gtgcagttgg ccatgagcga ccagttgacg gtctgcaggc cgggttgcac gacctctgag
9901 tacctgagcc gcgagaaggc gcgcgagtcg aagacatagt cgttgcaggt gcgcacgagg
9961 tactggtatc caactaggaa gtgcggcggc ggctggcggt agagcggcca gcgctgggtg
10021 gccggcgcgc ccggggccag gtcctcgagc atgaggcggt ggtagccgta gaggtagcgg
10081 gacatccagg tgatgccggc ggcggtggtg gaggcgcgcg ggaactcgcg gacgcggttc
10141 cagatgttgc gcagcggcag gaaatagtcc atggtcggca cggtctggcc ggtgagacgc
10201 gcgcagtcat tgacgctcta gaggcaaaaa cgaaagcggt tgagcgggct cttcctccgt
10261 agcctggcgg aacgcaaacg ggttaggccg cgtgtgtacc ccggttcgag tcccctcgaa
10321 tcaggctgga gccgcgacta acgtggtatt ggcactcccg tctcgacccg agcccgatag
10381 ccgccaggat acggcggaga gccctttttg ccgaccgagg ggagtcgcta gacttgaaag
10441 cggccgaaaa ccccgccggg tagtggctcg cgcccgtagt ctggagaagc tttgccaggg
10501 ttgagtcgcg gcagaacccg gttcgcggac ggccgcggcg agcgggactt ggtcaccccg
10561 ccgatttaaa gacccacagc cagccgactt ctccagttac gggagcgagc ccccttttt
10621 ctttttgcca gatgcatccc gtcctgcgcc aaatgcgtcc cacccccct ccggcgacca
10681 ccgcgaccgc ggccgtagca ggcgccggcg ctgtagcccc gccacagcag acagagatgg
10741 acttggaaga gggcgaaggg ctggcgagac tgggggcgcc gtccccggag cgacaccccc
10801 gcgtgcagct gcagaaggac gtgcgcccgg cgtacgtgcc tgcgcagaac ctgttcaggg
10861 accgcagcgg ggaggagccc gaggagatgc gcgactgccg ttttcgggcg ggcagggagc
10921 tgcgcgaggg cctggaccgc cagcgcgtgc tgcgcgacga ggatttcgag ccgaacgagc
```

FIG. 16A-3

```
10981 agacggggat cagccccgcg cgcgcgcacg tggcggcggc caacctggtg acggcctacg
11041 agcagacggt gaagcaggag cgcaacttcc aaaagagttt caacaaccat gtgcgcacgc
11101 taatcgcgcg cgaggaggtg gccctgggct tgatgcacct gtgggacctg gcggaggcca
11161 tcgtgcagaa cccggacagc aagcctctga cggcgcagct gttcctggtg gtgcagcaca
11221 gcagggacaa cgaggcgttc agggaggcgc tgctaaacat cgccgagccc gagggccgct
11281 ggctgctgga gctgatcaac atcttgcaga gcatcgtagt gcaggagcgc agcctgagcc
11341 tggccgagaa ggtggcggct atcaactact cggtgctgag cctgggcaag ttttacgcgc
11401 gcaagattta caagacgccg tacgtgccca tagacaagga ggtgaagata gacagctttt
11461 acatgcgcat ggcgctcaag gtgctgacgc tgagcgacga cctgggcgtg taccgcaacg
11521 accgcatcca caaggccgtg agcgcgagcc ggcggcgcga gctgagcgac cgcgagctga
11581 tgctgagtct gcgccgggcg ctggtagggg gcgccgccgg cggtgaggag tcctacttcg
11641 acatgggggc ggacctgcat tggcagccga gccggcgcgc cttggaggcc gcctacggtc
11701 cagaggactt ggatgaggat gaggaagagg aggaggatgc acccgctgcg gggtactgac
11761 gcctccgtga tgtgttttta gatgcagcaa gccccggacc ccgccataag ggcggcgctg
11821 caaagccagc cgtccggtct agcatcggac gactgggagg ccgcgatgca acgcatcatg
11881 gccctgacga cccgcaaccc cgagtccttt agacaacagc cgcaggccaa cagactctcg
11941 gccattctgg aggcggtggt cccctctcgg accaacccca cgcacgagaa ggtgctggcg
12001 atcgtgaacg cgctggcgga gaacaaggcc atccgtcccg acgaggccgg gctggtgtac
12061 aacgccctgc tggagcgcgt gggccgctac aacagcacaa acgtgcagtc caacctggac
12121 cggctggtga cggacgtgcg cgaggccgtg gcgcagcgcg agcggttcaa gaacgagggc
12181 ctgggctcgt tggtggcgct gaacgccttc ctggcgacgc agccggcgaa cgtgccgcgc
12241 gggcaggacg attacaccaa ctttatcagc gcgctgcggc tgatggtgac cgaggtgccc
12301 cagagcgagg tgtaccagtc gggcccagac tacttttcc agacgagccg gcagggcttg
12361 cagacggtga acctaagcca ggctttcaag aatctgcgcg ggctgtgggg cgtgcaggcg
12421 cccgtgggcg accggtcgac ggtgagcagc ttgctaacgc ccaactcgcg gctgctgctg
12481 ctgctgatcg cgcccttcac cgacagcggc agcgtgaacc gcaactcgta cctgggccac
12541 ctgctgacgc tttaccgcga ggccataggc caggcgcagg tggacgagca gaccttccag
12601 gagatcacta gcgtgagccg cgcgctgggt cagaacgaca ccgacagtct gagagccacc
12661 ctgaacttct tgctgacaaa tagacagcag aagattccgg cgcagtacgc gctgtcggcc
12721 gaggaggagc gcatcctgag atatgtgcag cagagcgtag ggcttttcct gatgcaggag
12781 ggggccaccc ccagcgccgc gctggacatg accgcgcgca acatggaacc tagcatgtac
12841 gccgccaacc ggccgttcat caataagctg atggactacc tgcaccgcgc ggctgccatg
12901 aactcggact actttactaa tgctatacta aacccgcact ggctcccgcc gccggggttc
12961 tacacgggcg agtacgacat gcccgacccc aacgatgggt tcctgtggga cgacgtggac
13021 agcgcggtgt tctccccgac cttgcaaaag cgccaggagg cggtacgcac gcccgcgagc
13081 gagggcgcgg tgggtcggag ccccttcct agcttaggga gtttgcatag cttgccgggc
13141 tcggtgaaca gcggcagggt gagccggccg cgcttgctgg gcgaggacga gtacctgaac
13201 gactcgctgc tgcagccgcc gcgggtcaag aacgccatgg ccaataacgg gatagagagt
13261 ctggtggaca aactgaaccg ctggaagacc tacgctcagg accataggga tgcgcccgcg
13321 ccgcggcgac agcgccacga ccggcagcgg ggcctggtgt gggacgacga ggactcggcc
13381 gacgatagca gcgtgttgga cttgggcggg agcggtgggg ccaacccgtt cgcgcatctg
13441 cagcccagac tggggcgacg gatgttttga atgaaataaa actcaccaag gccatagcgt
13501 gcgttctctt ccttgttaga gatgaggcgc gcggtggtgt cttcctctcc tcctccctcg
13561 tacgagagcg tgatggcgca ggcaaccctg gaggttccgt ttgtgcctcc gcggtatatg
13621 gctcctacgg agggcagaaa cagcattcgt tactcggaac tggctccgca gtacgacacc
13681 actcgcgtgt acttggtgga caacaagtcg gcggacatcg cttccctgaa ctaccaaaac
13741 gaccacagca acttcctgac cacggtggtg cagaacaacg atttcacccc cgccgaggcc
13801 agcacgcaga cgataaattt tgacgagcgg tcgcggtggg gcggtgattt gaagaccatt
13861 ctgcacacca acatgcccaa tgtgaacgag tacatgttca ccagcaagtt taaggcgcgg
13921 gtgatggtgg ctaggaaggt ggtagatcag aatgatagga gcaaggatga gttaaaatat
13981 gagtggtttg agtttaccct gcccgagggc aacttttccg agaccatgac catagacctg
14041 atgaacaacg ccatcttgga aaactacttg caagtggggc ggcaaaatgg cgtgctggag
14101 agcgatatcg gagtcaagtt tgacagcagg aatttcaagc tgggctggga cccggtaacc
14161 aagctggtga tgcctggggt ctacacctac gaggccttcc acccggacgt tgtgctgctg
14221 ccgggctgcg gggtggactt caccgagagc cgcctgagca acctcctggg cattcgcaag
14281 aagcaacctt tccaagaggg cttcaggatc atgtatgagg atctcgaggg tggtaacatc
14341 cccgccctcc tggatgtcaa gcaatatttg gatagtaaaa agaagcttga ggaggcaaca
14401 cagaatgcaa ccagggctgc tggagatatc agaggagaca gtcatattcc aagagctgtg
14461 gaacaagcgg ctgaaaagga tctggtcatt gtaccagtaa cacaagatga aagtaagaga
14521 agctataatg tcatagatgg cacccatgac accctctacc gaagttggta cctgtcctat
14581 acctacgggg accccgagaa gggggtgcag tcgtggacgc tgctcaccac cccggacgtc
```

FIG. 16A-4

```
14641 acctgcggcg cggagcaagt ctactggtcg ctgccggacc tcatgcaaga ccccgtcacc
14701 ttccgctcta cccagcaagt cagcaactac cccgtggttg gcgccgagct catgcccttc
14761 cgcgccaaga gcttttacaa cgacctcgcc gtctactccc agctcatccg cagctacacc
14821 tccctcaccc acgtcttcaa ccgcttcccc gacaaccaga tcctctgccg tccgcccgcg
14881 cccaccatca ccacggtcag tgaaaacgtg cctgctctca cagatcacgg gacgctaccg
14941 ctgcgcagca gtatccgcgg agtccagcga gtgaccgtca ctgacgcccg tcgccgcacc
15001 tgtccctacg tctacaaggc cctgggcata gtcgcgccgc gcgtgctttc cagtcgcacc
15061 ttctaaaaaa tgtctattct catctcgccc agcaataaca ccggctgggg tcttactagg
15121 cccagcacca tgtacggagg agccaagaag cgctcccagc agcaccccgt ccgcgtccgc
15181 ggccacttcc gcgctccctg gggcgcttac aagcgcgggc ggacttctac cgccgccgtg
15241 cgcaccaccg tcgacgacgt catcgactcg gtggtcgccg acgcgcgcaa ctatacccca
15301 gccccctcca ccgtggacgc ggtcatcgac agcgtggtgg ccgacgcgcg cgactatgcc
15361 agacgcaaga gccggcggcg acggatcgcc aggcgccacc ggagtacgcc cgccatgcgc
15421 gccgcccggg ctctgctgcg ccgcgccaga cgcacgggcc gccgggccat gatgcgagcc
15481 gcgcgccgcg ccgccactgc acccccccgca ggcaggactc gcagacgagc ggccgccgcc
15541 gctgccgcgg ccatttctag catgaccaga cccaggcgcg gaaacgtgta ctgggtgcgc
15601 gactccgtca cgggcgtgcg cgtgcccgtg cgcacccgtc ctcctcgtcc ctgatctaat
15661 gcttgtgtcc tcccccgcaa gcgacgatgt caaagcgcaa aatcaaggag gagatgctcc
15721 aggtcgtcgc cccggagatt tacggaccac cccaggcgga ccagaaaccc cgcaaaatca
15781 agcgggttaa aaaaaaggat gaggtggacg agggggcagt agagtttgtg cgcgagttcg
15841 ctccgcggcg gcgcgtaaat tggaaggggc gcagggtgca gcgcgtgttg cggcccggca
15901 cggcggtggt gtttacgccc ggcgagcggt cctcggtcag gagcaagcgt agctatgacg
15961 aggtgtacgg cgacgacgac atcctggacc aggcggcgga gcgggcgggc gagttcgcct
16021 acgggaagcg gtcgcgcgaa gaggagctga tctcgttgcc gctggacgag agcaacccca
16081 cgcctagcct gaagcccgtg accctgcagc aggtgctgcc ccaagcagtg ctgctgccga
16141 gccgcggggt caagcgcgag ggcgagaata tgtacccgac catgcagatc atggtgccca
16201 agcgccggcg cgtggaagaa gtgctggaca ccgtgaaaat ggatgtggag cccgaggtca
16261 aggtgcgccc catcaagcag gtggcgccgg gcctgggcgt gcagaccgtg gacattcaga
16321 tccccaccga catggatgtt gacaaaaaac cctcgaccag catcgaggtg cagaccgacc
16381 cctggctccc agcctccacc gctgccgtct ccacttctac cgccgccacg gctaccgagc
16441 ctcccagaag gcgaagatgg ggccctgcca accggctgat gcccaactac gtattgcatc
16501 cttccattat cccgacgccg ggctatcgcg gcacccggta ctacgccagc cgcaggcgcc
16561 cagccagcaa acgccgccgc cgcaccgcca cccgccgccg tctggccccc gcccgcgtgc
16621 gccgcgtaac cacgcgccgg ggccgctcgc tcgttctgcc caccgtgcgc taccacccca
16681 gcatccttta atccgtgtgc tgtgatactg ttgcagagag atggctctca cttgccgcct
16741 gcgcatcccc gtcccgaatt accgaggaag atcccgccgc aggagaggca tggcaggcag
16801 cggcctcaac cgccgccggc ggcgggccat gcgcaggcgc ctgagtggcg gctttctgcc
16861 cgcgctcatc cccataatcg cggcggccat cggcacgatc ccgggcatag cttccgttgc
16921 gctgcaggcg tcgcagcgcc gttgatgtgc gaataaagcc tctttagact ctgacacacc
16981 tggtcctgta tatttttaga atggaagaca tcaattttgc gtccctggct ccgcggcacg
17041 gcacgcggcc gttcatgggc acctggaacg agatcggcac cagccagctg aacgggggcg
17101 ccttcaattg gagcagtgtc tggagcgggc ttaaaaattt cggctcgacg ctccggacct
17161 atgggaacaa ggcctggaat agtagcacgg ggcagttgtt aagggaaaag ctcaaagacc
17221 agaacttcca gcagaaggtg gtggacggcc tagcctcggg cattaacggg gtggtggaca
17281 tagcaaacca ggccgtgcag cgcgagataa acagccgcct ggacccgcgg ccgcccacgg
17341 tggtggagat ggaagatgca actcctccgc cgcccaaggg cgagaagcgg ccgcggcccg
17401 acgcggagga gacgatcctg caggtggacg agccgccctc gtacgaggag gccgtcaagg
17461 ccggcatgcc caccacgcgt atcatcgcgc cactggccac tggtgtaatg aaacccgcca
17521 cccttgacct gcctccgcca cccacgcccg ctccaccgaa ggcagctccg gttgtgcagc
17581 cccctcctgt ggcgaccgcc gtgcgccgcg tccccgcccg ccgccaggcc cagaactggc
17641 agagcacgct gcacagtatc gtgggcctgg gagtgaaaag tctgaagcgc cgccgatgct
17701 attgagagag aggaaagagg acactaaagg gagagcttaa cttgtatgtg ccttaccgcc
17761 agagaacgcg cgaagatggc taccccctcg atgatgccgc agtgggcgta catgcacatc
17821 gccgggcagg acgcctcgga gtacctgagc ccgggtctgg tgcagtttgc ccgcgccacc
17881 gacacgtact tcagcctggg caacaagttt aggaacccca cggtggctcc cacccacgat
17941 gtgaccacgg accggtccca gcgtctgacg ctgcgctttg tgcccgtgga tcgcgaggac
18001 accacgtact cgtacaaggc gcgcttcact ctggccgtgg gcgacaaccg ggtgctagac
18061 atggccagca cttactttga catccgcggc gtcctggacc gcggtcccag cttcaaaccc
18121 tactcgggca cggcttacaa cagcctggcc cccaaaggcg cccccaactc tagtcagtgg
18181 gaacaagcta aagctaccaa tgccggtcaa aaggaaactc acacatttgg agtagccgct
18241 atgggcggag aagacattac agtgaaaggt cttcaaattg gaactgatga aactaaggaa
```

FIG. 16A-5

```
18301 gatggagagg atgaaatttt tgcagatcaa acattccagc cagaacctca agtgggagaa
18361 cagaactggc aagaaacgtt tgttttctat ggaggcagag ctcttaagaa agaaaccaaa
18421 atgaagccat gttatggctc ttatgcgaga cccacaaatg aaaagggagg acaggctaaa
18481 tttacacttg atgaaaaagg tcagccaacc aaaattcctg atattacaat ggatttcttt
18541 gatagtccac aagatgatac atcaggtgta actaataagc cagatattgt catgtatgca
18601 gaaaatgtaa atttagaagc tcctgacaca catgtagttt acaaaccagg caaagatgat
18661 tctagttctt ccgctaacct cacacaacag gccatgccta acagaccgaa ctacatcggg
18721 ttcagagaca actttgtggg tcttatgtac tacaatagta ctggcaacat gggtgtgctg
18781 gctggtcagg cctctcagtt gaatgctgtg gtcgacttgc aagacagaaa caccgagctg
18841 tcttaccagc tattgctaga ttctctgggt gacagaacca gatactttag catgtggaat
18901 tctgcagtgg acagctatga ccccgatgtc aggatcattg agaatcacgg tgtggaagat
18961 gaacttccaa actattgctt cccactgaat ggcagtggtt ctaacagcac atacaaaggt
19021 gttaaagctg gaactggaaa caattgggat gacgatgaaa atgttgcaag acaaaatcag
19081 attggcactg gcaacctgtt cgccatggag atcaacctcc aggccaacct atggaagagt
19141 tttctgtact cgaacgtggc cctgtacctg cccgactcct acaagtacac gccggccaac
19201 gtcacgctgc ccaccaacac caacacctac gactacatga acggccgcgt ggtagccccc
19261 tcgctggtgg acgcctacat caacattggc gcccgctggt cgctggaccc catggacaat
19321 gtcaatccct tcaaccacca ccgcaacgcg ggcctgcgct accgctccat gctcctgggc
19381 aacggccgct acgtgccctt ccacatccaa gtgccccaaa agttctttgc catcaagaac
19441 ctgcttctgc tccccggttc ctacacctac gagtggaact tccgcaagga cgtcaacatg
19501 atcctgcaga gttccctcgg caacgacctg cgcgtcgacg gcgcctccgt ccgcttcgac
19561 agcgtcaacc tctacgccac cttcttcccc atggcgcaca acaccgcctc caccctggaa
19621 gccatgctgc gcaacgacac caacgaccag tccttcaacg actacctctc ggccgccaac
19681 atgctctacc ccatcccggc caaggccacc aacgtgccca tctccatccc ctcgcgcaac
19741 tgggccgcct tccgcggctg gagtttcacc cggctcaaga ccaaggaaac tccctccctc
19801 ggctcgggtt tcgaccccta ctttgtctac tcgggctcca tcccctacct cgacgggacc
19861 ttctacctca accacacctt caagaaggtc tccatcatgt tcgactcctc ggtcagctgg
19921 cccggcaacg accggctgct cacgccgaac gagttcgaga tcaagcgcag cgttgacggg
19981 gagggctaca acgtggccca atgcaacatg accaaggact ggttcctcgt ccagatgctc
20041 tcccactaca acatcggcta ccagggcttc cacgtgcccg agggctacaa ggaccgcatg
20101 tactccttct tccgcaactt ccagcccatg agcaggcagg tggtcgatga gatcaactac
20161 aaggactaca aggccgtcac cctacccttc cagcacaaca actcgggctt caccggctac
20221 cttgcgccca ccatgcgcca ggggcagccc taccccgcca acttccccta cccgctcatc
20281 ggctccaccg cagttccctc cgtcacccag aaaaagttcc tctgcgacag ggtcatgtgg
20341 cgcatcccat tctccagcaa ctttatgtcc atgggcgccc tcaccgacct gggtcagaac
20401 atgctctatg ccaactcggc ccacgcgctc gacatgacct ttgaggtgga ccccatggat
20461 gagcccaccc tcctctatct tctcttcgaa gttttcgacg tggtcagagt gcaccagccg
20521 caccgcggcg tcatcgaggc cgtctacctg cgcacgccct tctccgccgg caacgctacc
20581 acttaagcat gagcggctcc agcgaacaag agctcgcggc catcgtgcgc gacctgggat
20641 gcgggcccta ctttttggga acccacgaca agcgcttccc tggcttcctt gccggcgaca
20701 agctggcctg cgccatcgtc aacacggccg gccgcgagac cggaggcgtg cactggctcg
20761 cctttggctg gaatccgcgc tcgcgcacct gctacatgtt cgaccccttt gggttctcgg
20821 accgccggct caagcagatt tacagcttcg agtacgaggc catgctgcgc cgaagcgcgc
20881 ttgcctcctc gcccgaccgc tgtctcagcc tcgagcagtc cacccagacc gtgcaggggc
20941 ccgactccgc cgcctgcgga ctttttttgtt gcatgttttt gcatgccttc gtgcactggc
21001 ccgaccgacc catggacgga aaccccacca tgaacttgct gacgggggtg ccaaacggca
21061 tgctacaatc gccacaggtg ctgcccaccc tcaggcgcaa ccaggaggag ctctaccgct
21121 tcctcgcgcg ccactcccct tactttcgat cccaccgcgc cgccatcgaa aacgccaccg
21181 cttttgataa aatgaaacaa ctgcgtgtat ctcaataaac agcactttat tttacatgca
21241 ctggagtata tgcaagttat ttaaaagtcg aaggggttct cgcgctcgtc gttgtgcgcc
21301 gcgctgggga gggccacgtt gcggtactgg tacttgggaa gccacttgaa ctcggggatc
21361 accagtttgg gcactggggt ctcggggaag gtctcgctcc acatgcgccg gctcatctgc
21421 agggcgccca gcatgtccgg gccggagatc ttgaaatcac aattggggcc ggtgctctgc
21481 gcgcgcgagt tgcggtacac ggggttgcag cactggaaca ccattagact ggggtacttc
21541 acactggcaa gcacgctctt gtcgctgatc tgatccttgt ccaggtcctc ggcgttgctc
21601 aggccgaacg gggtcatctt gcacagctgg cggcccagga agggcacgct ctgaggcttg
21661 tggttacact cgcagtgcac gggcatcagc atcatccccg cgccgcgctg catattcggg
21721 tagagggcct tgacgaaggc cgtgatctgc ttgaaagctt gctgggcctt agccccctcg
21781 ctgaaaaaca ggccgcagct cttcccgcta aactggttat tcccgcaccc ggcatcatgc
21841 acgcagcagc gcgcgtcatg gctggtcagt tgcaccacgc tacgtcccca gcggttctgg
21901 gtcaccttgg ccttgctggg ctgctccttc aacgcgcgct gcccgttctc gctggtcaca
```

FIG. 16A-6

```
21961 tccatctcca ccacgtggtc cttgtggatc atcaccgtcc catgcagaca cttgagctga
22021 ccctcgacat cgcagcagcc atgatcccac agggcgcagc cggtgcactc ccagttctta
22081 tgcgcgatcc cgctgtggct gaagatgtaa ccttgcaaca ggcgacccat gacggtgcta
22141 aatgctttct gggtggtgaa ggtcagttgc agaccgcggg cctcctcgtt catccaggtc
22201 tggcacatct tttggaagat ctcggtctgc tcgggcatga gcttgtaagc atcgcgcagg
22261 ccgctgtcga cgcggtagcg ttccatcagc acgttcatgg tatccatgcc cttctcccag
22321 gacgagacca gaggcagact caggggttg cgcacgttca ggacaccggg ggtcgcaggc
22381 tcgacgatgc gttttccgtc cttgccttcc ttcaacagaa ccggaggctg gctgaatccc
22441 actcccacga ttacggcatc ttcctggggc atctcttcgt cggggtctac cttggtcaca
22501 tgcttggtct ttctggcttg cttctttttt ggagggctgt ccacggggac cacgtcctcc
22561 tcggaagacc cggagcccac ccgctgatac tttcggcgct tggtgggcag aggaggtggt
22621 ggcggcgagg ggctcctctc ctgctccggc ggatagcgcg ccgacccgtg gccccggggc
22681 ggagtggcct ctcgctccat gaaccggcgc acgtcctgac tgccgccggc cattgtttcc
22741 taggggaaga tggaggagca gccgcgtaag caggagcagg aggaggactt aaccacccac
22801 gagcaaccca aaatcgagca ggacctgggc ttcgaagagc cggctcgtct agaaccccca
22861 caggatgaac aggagcacga gcaagacgca ggccaggagg agaccgacgc tgggctccag
22921 catggctacc tgggaggaga ggaggatgtg ctgctaaaac acttgcagcg ccaatccatc
22981 atcctccggg acgccctggc cgaccggagc gaaacccctc tcagcgtcga ggagctgtgt
23041 cgggcctacg agctcaacct cttctcgccg cgcgtgcccc ccaaacgcca gcccaacggc
23101 acctgcgagc ccaacccgcg tctcaacttc tatcccgtct ttgcggtccc cgaggcccta
23161 gccacctatc acatcttttt caagaaccaa aagatccccg tctcctgccg cgccaaccgc
23221 acccgcgccg acgcgctcct cgctctgggg cccggcgcgc gcatacctga tatcgcttcc
23281 ctggaagagg tgcccaagat cttcgaaggg ctcggtcggg acgagacgcg cgcggcaaac
23341 gctctgaaag aaacagcaga ggaagagggt cacactagcg ccctggtaga gttggaaggc
23401 gacaacgcca ggctggccgt gctcaagcgc agcgtcgagc tcacccactt cgcctacccc
23461 gccgtcaacc tcccgcccaa ggtcatgcgt cgcatcatgg atcagctcat catgccccac
23521 atcgaggccc tcgatgaaag tcaggagcag cgccccgagg acgcccggcc cgtggtcagc
23581 gacgagcagc tcgcgcgttg gctcgggacc cgcgaccccc aggctttgga acagcggcgc
23641 aagctcatgc tggccgtggt cctggtcacc ctcgagctcg aatgcatgcg ccgcttcttc
23701 agcgaccccg agaccctgcg taaggtcgag gagaccctgc actacacttt caggcacggt
23761 ttcgtcaggc aggcctgcaa gatctccaac gtggagctga ccaacctggt ctcatgcctg
23821 gggatcctgc acgagaaccg cctgggacag accgtgctcc actctactct gaagggcgag
23881 gcgcgtcggg actatgtccg cgactgtgta tttctcttta tctgccacac ctggcaagca
23941 gccatgggcg tgtggcagca gtgtctcgag gacgaaaatc tgaaggagct ggacaagctt
24001 cttgctagaa accttaaaaa gctgtggacg ggcttcgacg agcgcaccgt cgcctcggac
24061 ctggccgaga tcgtttttcc agaacgcctg aggcagacgc tgaaaggcgg gctgcccgac
24121 ttcatgagcc agagcatgtt gcaaaactac cgcactttca ttctcgagcg atctgggatg
24181 ctacccgcca cctgcaacgc attcccctcc gactttgtcc cgctgagcta ccgcgagtgt
24241 cccccgccgc tgtggagcca ctgctatctc ttgcagctgg ccaactacat cgcctaccac
24301 tcggacgtga tcgaggacgt gagcggcgag gggcttctcg agtgccactg ccgctgcaac
24361 ctgtgctccc cgcaccgctc cctggtctgc aacccccagc ttctgagcga gacccaggtc
24421 atcggtacct tcgagctgca aggtccgcag gagtccaccg ctccgctgaa actcacgccg
24481 gggttgtgga cttccgcgta cctgcgcaaa tttgtacccg aggactacca cgcccatgaa
24541 ataaagttct tcgaggacca atcgcgccca cagcacgcgg atctcacggc ctgcgtcatc
24601 acccagggcg cgatcctcgc ccaattgcac gccatccaaa aatcccgcca agagtttctt
24661 ctaaaaaagg gtagaggggt ctacctggac ccccagacgg gcgaggtgct caacccgggt
24721 ctcccccagc atgccgagga agaagcagga gccgctagtg gagcagatgg aagaagaatg
24781 ggacagccag gcagaggagg acgaatggga ggaggagaca gaggaggaag aattggaaga
24841 ggtggaagag gagcaggaaa cagagcagcc cgtcgccgca ccatccgcgc cggcagcccc
24901 gccggtcacg gatacaacct ccacagctcc ggccaagcct cctcgtagat gggatcgagt
24961 gaagggtgac ggtaagcacg agcggcaggg ctaccgatca tggagggtcc acaaagccgc
25021 gatcatcgcc tgcttgcaag actgcggggg gaacatcgct ttcgcccgcc gctacctgct
25081 cttccaccgc ggggtgaaca tcccccgcaa cgtgttgcat tactaccgtc accttcacag
25141 ctaagaaaaa gcaagtaaga ggagtcgccg gaggaggcct gaggatcgcg gcgaacgagc
25201 cctcgaccac cagggagctg aggaaccgga tcttccccac tctttatgcc atttttcagc
25261 agagtcgagg tcagcagcaa gaactgaaag taaaaaaccg gtctctgcgc tcgctcaccc
25321 gcagttgctt gtaccacaaa aacgaagatc agctgcagcg cactctcgaa gacgccgagg
25381 ctctgttcca caagtactgc gcgctcactc ttaaagacta aggcgcgccc acccggaaaa
25441 aaggcgggaa ttacctcatc gccaccatga gcaaggagat tcccacccct tacatgtgga
25501 gctatcagcc ccagatgggc ctggccgcgg gcgcctccca ggactactcc acccgcatga
25561 actggctcag tgccggcccc tcgatgatct cacgggtcaa cggggtccgt aaccatcgaa
```

FIG. 16A-7

```
25621 accagatatt gttggagcag gcggcggtca cctccacgcc cagggcaaag ctcaacccgc
25681 gtaattggcc ctccaccctg gtgtatcagg aaatccccgg gccgactacc gtactacttc
25741 cgcgtgacgc actggccgaa gtccgcatga ctaactcagg tgtccagctg gccggcggcg
25801 cttcccggtg cccgctccgc ccacaatcgg gtataaaaac cctggtgatc cgaggcagag
25861 gcacacagct caacgacgag ttggtgagct cttcgatcgg tctgcgaccg gacggagtgt
25921 tccaactagc cggagccggg agatcgtcct tcactcccaa ccaggcctac ctgaccttgc
25981 agagcagctc ttcggagcct cgctccggag gcatcggaac cctccagttc gtggaggagt
26041 ttgtgccctc ggtctacttc aaccccttct cgggatcgcc aggcctctac ccggacgagt
26101 ttataccgaa cttcgacgca gtgagagaag cggtggacgg ctacgactga atgtcccatg
26161 gtgactcggc tgagctcgct cggttgaggc atctggacca ctgccgccgc ctgcgctgct
26221 tcgcccggga gagctgcgga ctcatctact ttgagtttcc cgaggagcac cccaacggcc
26281 ctgcacacgg agtgcggatc accgtagagg gcaccaccga gtctcacctg gtcaggttct
26341 tcacccagca acccttcctg gtcgagcggg accggggagc taccacctac accgtctact
26401 gcatctgtcc taccccgaag ttgcatgaga atttttgctg tactctttgt ggtgagttta
26461 ataaaagctg aactaagaac cttctttgga atcccttgtc atcatcaaat caacaagacc
26521 atcaacttca cctttgagga acaggtgaac tttacctgca agccacacaa gaagtacatc
26581 atctggtttt atcacaacac tactctagca gtagccaaca cctgctcgaa cgacggtgtt
26641 ctcctaccta acaatctcac cagtggacta accttctcag ttaaaagggc aaagctaatt
26701 cttcatcgcc ctattgtaga aggaacttac cagtgtcaga gcggaccttg cttccacagt
26761 ttcactttgg tgaacgttac cggcagcagc acagccgctc cagaaacatc taaccttctt
26821 tctgatacta acaaacctcg tgtcggaggt gagctttggg ttccatctct aacagagggt
26881 gggagttcta ttgaagtggt tgggtatttg attttagggg tggtcattgg tgggtgcata
26941 gcagtgctgt atcaacttcc ttgctgggtc gaaatcaggg tatttatctg ctgggtcaga
27001 cattgtgggg aggaaccatg aaggggctct tgctgattat cctttccctg gtgggggtg
27061 tgctgtcatg ccacgaacag ccacgatgta acattaccac aggcaatgag aggaacgact
27121 gctctgtagt tatcaaatgc gagcaccatt gtcctctcaa catcacattc aagaataaga
27181 ccatgggaaa tgtatgggtg ggattctggc aaccaggaga tgagcagaac tacacggtca
27241 ctgtccatgg tagcgatggc aatcacactt tcggtttcaa attcatttt gaagtcatgt
27301 gtgatatcac actacatgtg gctagacttc atggcttgtg gccccctacc aaggagaaca
27361 tggtgggttt ttctttggct tttgtgatca tggcctgctt gatgtcaggt ctgctggtag
27421 gggctctagt gtggtttctg aaacgcaagc ccaggtacgg aaatgaggag aaggaaaaat
27481 tgctataaat tctttttctc ttcgcacaac catgaataca gtgttccgta tcgtgctgct
27541 ctctcttctt gtagctttcg gtcaggcagg aattcatatt attaatgcta catggtggga
27601 taatataact ttagtgggac cctcagatac tccagttacc tggtatgatg gcaagggatt
27661 gcaatttttgt gacggaagta cagttaagaa tccgcagatc agacatactt gtaatgatca
27721 aaacttaact ctgattcatg ttaacaaaac ccatgaaaga acatacatgg gttacagaca
27781 tgacagtaag ggaaaagtag actataaggt tacagtcatt ccacctcctc ctgctactgt
27841 aaagccacaa ccagatccag aaaatgtctt tgtttatatg ggaaataatg taactttagt
27901 tggacctcca ggaattccag ttagttggta ttatcataat ggcacacagt tctgcgatgg
27961 agataaaatt attcatccag aattcaacca cacctgtgat aaacaaaacc ttacactgct
28021 gtttgtaaac tttacacatg atggaggcta tcttggattc aattacaaag gtactcagag
28081 aattcagtat gaggttatag ttttagatcg atttccaaat tctggtcaga tgaaaattga
28141 agaacaaagt gaggaaacag aacagaaaca tactgagcat aataaggctg gacaaaagca
28201 gggtatagat acaaatcaaa agaaagctaa taacagacaa aagccatctc aaaggccatc
28261 aagaagacgg ccgacaaaca ctcctgagac aaaacaactt acagtgtcta ttgggtctaa
28321 cttaacttta gttggtccag atggaaaagt cacttggtat gatggtgatt taaaaagacc
28381 atgtgaagaa caaaactata ggcttccaca tcagtgtagt gctcagaact taactttaat
28441 taatgtaact aaatctcatg agggaactta ctatggcact aatgacaaag acgaaagcaa
28501 aagatacaga gtgaaagtga acactacaaa ttctcaagct gtaaaaatta acccatatac
28561 cagacctact actcctgatc agaaacacag atttgaatta caaattgaaa ataatgcaaa
28621 tgatgaagaa tcaaaaattc catctactac tgtggcaatc gtggtgggag tgattgcggg
28681 cttcataact ataatcattg tcattctgtg ctacatctgc tgccgcaagc gtcccagggc
28741 atacaatcat atggtagacc cactactcag cttctcttac tgagactcag tcactttcat
28801 ttcagaacca tgaaggcttt cacagcttgc gttctgttta acataatcac acttagtgta
28861 gctgcaaatg gttttaaaca tgttaatgtt accagattaa gtaatgtaac actgacagga
28921 gctggaatta atactacatg gacagggtat tttaatgagg gtccaaaagg aaaaaatggg
28981 tggatgaata tttgcacatg gggcgatcct agatatgtgt gccatggaaa tagcagtact
29041 attactaatc ttacagttgt ggcacttcta aatttaacca ctaacagaag atttaaagca
29101 gaaagtttta ctagtaacga tggttatgaa actaccagtg caaaatttta tgaaattaaa
29161 attattgagc ttccaacaac tagagcaccc accacagtta ggacaacaca gcctaccact
29221 gtgcccacta cacatccaac caccacagtc agtacaacta ttgagaccac tactcatact
```

FIG. 16A-8

```
29281 acacagctag acacaacagt gcagaatact actttattga ttgggttttt actgagagga
29341 aatgaaagta ctactgaaca gacagaggct acctcaagtg ccttcagcag cactgcaaat
29401 ttaacttcgc ttgcttggac taatgaaacc ggagtatcat tgatgaatcg acagccttac
29461 tcaggtttgg atattcaaat tacttttctg gttgtctgtg ggatctttat tcttgcggtt
29521 cttctgtact ttgtctgctg caaagccaga gagaaatcta ggcggcccat atacaggcca
29581 gtaatcgggg aacctcagcc tctccaagtg gatggaggct taaggaatct tctcttctct
29641 tttacagtat ggtgatcagc catgattcct aggttcttcc tatttaacat cctgttctgt
29701 ctcttcaaca tctgtgctgc cttcgcggcc gtctcgcacg cctcgcccga ctgtctaggg
29761 cctttcccaa catacctcct ctttgccctg ctaacctgca cctgcgtctg cagcattgtc
29821 tgcgtggtca tcacctttct gcagctcatc gactggtgct gcgcgcgcta caattatctc
29881 caccacagtc ccgaatacag ggacgagaac gtagccagaa tcttaaggct catctgacca
29941 tgcagcctct gctcatgctg atatccctcc tatcccctgc ccttgccact tctgctgatt
30001 actctaaatg caaattcgcg gacatatgga atttcttaga ttgctatcag gagaaaattg
30061 atatgccctc ctattacttg gtgattgttg gggtagtcat ggtctgctca tgcactttct
30121 ttgccattat gatctacccc tgttttaatc ttggctggaa ctctgttgag gcattcacat
30181 acacactaga aaacagttca ctagcctcca cgccaccacc cacaccgcct ccccgcagaa
30241 atcagttccc tatgattcag tacttagaag agccccctcc ccggcccccc tccactgtta
30301 gctactttca cataaccggc ggcgatgact gaccacctgg acctcgagat ggacggccag
30361 gcctccgagc agcgcatcct gcaactgcgc gtccgacagc agcaggagcg ggccgccaag
30421 gagctcctcg atgccatcaa catccaccag tgcaagaagg gcatcttctg cctggtcaag
30481 caggcaaaga tcacctacga gctcgtgtcc ggcggcaagc agcatcgcct cgcctatgag
30541 ctaccccagc agaagcaaaa gttcacctgc atggtgggcg tcaaccccat agtcatcacc
30601 cagcagtcgg gcgagaccaa cggctgcatc cactgctcct gcgaaagccc cgagtgcatc
30661 tactccctcc tcaagaccct ttgcggactc cgcgacctcc tccccatgaa ctgatgttga
30721 ttaaaagccc aaaaaccaat caaacccttc cccaattact cataagaata aatcattgga
30781 actaatcatt caataaagat cacttacttg aaatctgaaa gtatgtctct ggtgtagttg
30841 ttcagcagca cctcggaacc ctcctcccag ctctggtact ccagtccccg gcgggcggcg
30901 aacttcctcc acaccttgaa agggatgtca aattcctggt ccacaatttt cattgtcttc
30961 cctcagatga caaagaggct ccgggtggaa gatgacttca accccgtcta cccctatggc
31021 tacgcgcgga atcagaatat ccccttcctt actccccccc ttgtttcttc cgatggattc
31081 caaaacttcc cacctggggt cctgtcactc aaactggctg acccaatcgc catcactaat
31141 ggggatgttt cactcaaggt gggagggggt cttactgttg aaaaagatag tggaaatcta
31201 aaggtgaacc ctaaggctcc cttgcaagtt acaactgata aacagttgga aattgcactg
31261 gcttatccat ttgaagtcag taatggcaag cttggcataa aagcaggtca tggattgaaa
31321 gtcattgaca aaattgctgg tttggaaggt ttggcaggta cgcttgtagt tttgactgga
31381 aaaggaatag gtactgaaaa tcttgaaaac agtgatgggt caagtagagg agttggtata
31441 aacgtaagac ttgctaaaga tggaggtctg tcttttgata aaaagggtga tttagttgct
31501 tggaataaac atgatgacag acgcactcta tggacaactc ccgacccatc cccaaattgt
31561 acaatcgatc aggaaaggga ttcaaagctc actttagtat taacaaaatg tggcagtcaa
31621 attttggcta atgtctcttt acttgttgta aaaggaaaat ttagtaacat aaacaataat
31681 actaatccaa ctgataaaaa aatcacagta aagctacttt ttaatgaaaa gggagtatta
31741 atggacagtt cgacacttaa gaaagaatat tggaactaca gaaatgataa ttctactgta
31801 tctcaggcct atgataatgc agttcctttt atgccaaaca taaaagctta tcctaaacct
31861 accacagaca cttcggctaa accagaagat aaaaaaagtg ctgctaaaag atacattgtg
31921 agcaatgtct atattggagg cttgccagat aaaactgttg ttataactat taagtttaat
31981 gcagaaactg aatgtgctta ttcgattacc tttgaattca catgggcaaa aacctttgaa
32041 gatgtgcagt ttgattcctc ctcttttacc ttttcctata ttgcccaaga aaatgaggac
32101 gaagacaaat aaaatgtttt aaaatgaatt catgtatctt tattgatttt tacaccagca
32161 cgggtagtca gtctcccacc accagcccat ttcacagtgt aaacgattct ctcagcacgg
32221 gtggccttaa atagggaaat gttctgatta gtgcgggaac tggacttggg gtctataatc
32281 cacacagttt cctggcgagc caaacggggg tcggtgattg agatgaagcc gtcctctgaa
32341 aagtcatcca agcgggcctc acagtccaag gtcacagtct ggtgaaacga gaagaacgca
32401 cagattcata ctcggaaaac aggatgggtc tgtgcctctc catcagcgcc ctcaacagtc
32461 tctgccgccg gggctcggtg cggctgctgc agatgggatc gggatcacaa gtctctctga
32521 ctatgatccc cacagccttc agcatcagtc tcctggtgcg tcgggcacag caccgcatcc
32581 tgatctcgct catgttctca cagtaagtgc agcacataat caccatgtta ttcagcagcc
32641 cataattcag ggtgctccag ccaaaactca tgttggggat gatggaaccc acgtgaccat
32701 cgtaccagat gcggcagtat atcagatgcc tgcccctcat gaacacactg cccatataca
32761 tgatctcttt gggcatgtct ctgttcacaa tctgacggta ccagggaaag cgctggttga
32821 acatgcaccc gtaaatgact ctcctgaacc acacggccag cagggtgcct cccgcccgac
32881 actgcaggga gcccggggat gaacagtggc aatgcaggat ccagcgctcg tacccgctca
```

FIG. 16A-9

32941 ccatctgagc tctcaccaag tccagggtag cggggcacag gcacactgac atacatcttt
33001 ttaaaatttt tatttcctct ggagtcaaga tcatatccca ggggactgga aactcttgga
33061 gcagggtaaa gccagcagca catggtaatc cacggacaga acttacatta tgataatctg
33121 catgatcaca atcaggcaac aggggatgtt gttcagtcag tgaagccctg gtttcctcat
33181 cagatcgtgg taaacgggcc ctgcgatatg gatgatggcg gagcgagctg gattgaatct
33241 cggtttgcat tgtagtggat tctcttgcgt accttgtcgt acttctgcca gcagaaatgg
33301 gcccttgaac agcagatacc cctcctgcgg ccgtcctttc gctgctgccg ctcagtcatc
33361 caactgaagt acatccattc tcgaagattc tggagaagtt cctctgcatc tgatgaaaca
33421 aaaaacccgt ccatgcgaat tcccctcatc acatcagcca ggactctgta ggccatcccc
33481 atccagttaa tgctgccttg tctatcattc agaggggggcg gtggcaggat tggaagaacc
33541 atttttattc caaacggtct cgaaggacga taaagtgcaa gtcacgcagg tgacagcgtt
33601 cccctccgct gtgctggtgg aaacagacag ccaggtcaaa acccactcta ttttcaaggt
33661 gctcgaccgt ggcttcgagc agtggctcta cgcgtacatc cagcataaga atcacattaa
33721 aggctggccc tccatcgatt tcatcaatca tcaggttaca ttcctgcacc atccccaggt
33781 aattctcatt tttccagcct tggattatct ctacaaattg ttggtgtaag tccactccgc
33841 acatgtggaa aagctcccac agtgcccccct ccactttcat aatcaggcag accttcataa
33901 tagaaacaga tcctgctgct ccaccacctg cagcgtgttc aaaacaacaa gattcaataa
33961 ggttctgccc tccgccctga gctcgcgcct caatgtcagc tgcaaaaaat cacttaagtc
34021 ctgggccact acagctgaca attcagagcc agggctaagc gtgggactgg caagcgtaag
34081 ggaaaacttt aatgctccaa agctagcacc caaaaactgc atgctggaat aagctctctt
34141 tgtgtctccg gtgatgcctt ccaaaatgtg agtgataaag cgtggtagtt tttctttaat
34201 catttgcgta atagaaaagt cctgtaaata agtcactagg accccaggga ccacaatgtg
34261 gtagcttaca ccgcgtcgct gaagcatggt tagtagagat gagagtctga aaaacagaaa
34321 gcatgcacta aactaaggtg gctattttca ctgaaggaaa aatcactctc tccaacaaca
34381 gggtacccac tgggtggccc ttgcggacat acaaaaatcg gtccgtgtga ttaaaaagca
34441 gcacagtaag ttcctgtctt cttccggcaa aaatcacatc ggactgggtt agtatgtccc
34501 tggcatggta gtcattcaag gccataaatc tgccctgata tccagtagga accagcacac
34561 tcacttttag gtgaagcaat accacccccat gcggaggaat gtggaaagat tcagggcaaa
34621 aaaaattata tctattgcta gtcccttcct ggacgggagc aatccctcca ggactatcta
34681 tgaaagcata cagagattca gccatagctc agcccgctta ccagtagaca gagagcacag
34741 cagtacaagc gccaacagca gcgactgact acccactgac ccagctccct atttaaaggc
34801 gccttacact gacgtaatga ccaaaggtct aaaaaccccg ccaaaaaaaa acacacacgc
34861 cctgggtgtt ttttgcgaaa acacttccgc gttctcactt cctcgtattg atttcgtgac
34921 ttaacttccg ggttcccacg ttacgtcact tctgcccttа catgtaactc agtcgtaggg
34981 cgccatcttg cccacgtcca aaatggcttc catgtccagc cacgcctccg cggcgaccgt
35041 tagccgtgcg tcgtgacgtc atttgcatca tcttctctcg tccaatcagc gctggccccg
35101 ccctaaattc aaaagctcat ttgcatgtta acttttgttt actttgtggg gtatattatt
35161 gatgatc

SEQ ID NO: 5

FIG. 16A-10

Grp	Vaccine at Wk 0, Wk 4	Monkey ID	Pre		Wk 4		Wk 8		Wk 12	
			Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
1	Ad24ΔE1gogΔOrf6Ad5Orf6 10^11 vp	00C072	3	4	4	381	3	150	3	68
		00C178	3	3	1	559	1	743	0	635
		00C222	0	3	1	369	1	753	0	670
		00D011	1	9	9	211	4	273	0	520
		00D023	0	6	0	295	1	459	1	368
		00D031	15	5	10	103	1	101	1	40
2	Ad24ΔE1gogΔOrf6Ad5Orf6 10^10 vp	99C168	4	6	0	118	5	241	3	209
		99C170	10	5	5	241	3	141	3	103
		99C173	1	3	0	23	0	14	0	21
3	Ad24ΔE1gogΔE4Ad5Orf6 10^10 vp	99C154	0	3	0	93	0	60	1	53
		99C158	1	0	1	141	0	101	1	120
		99C177	0	0	0	45	0	39	0	79
4	MRKAd5-HIVgag 10^11 vp	00C018	1	5	13	1025	0	824	3	753
		00C034	0	4	5	219	5	404	0	491
		00C058	4	4	3	1086	0	440	0	439
5	MRKAd5-HIVgag 10^10 vp	99C218	0	3	5	2500	0	1580	10	1655
		99C227	6	1	4	529	5	365	5	1004
		99D185	ND	ND	0	425	0	310	0	271

FIG. 17

Vaccine at Wk 0, Wk 4	Monkey ID	Gag-Specific (Wk 12)	
		%CD4	%CD8
Ad24ΔE1gagΔOrf6Ad5Orf6 10^11 vp	00C072	0.02	0.02
	00C178	0.05	0.38
	00C222	0.02	0.40
	00D011	0.02	0.27
	00D023	0.01	0.11
	00D031	0.01	0.01
MRKAd5-HIVgag 10^11 vp	00C018	0.05	0.41
	00C034	0.06	0.18
	00C058	0.02	0.28

FIG. 18

Grp	Vaccine at Wk 0, Wk 4	Monkey ID	Wk 4	WK 8
1	Ad24ΔE1gagΔOrf6Ad5Orf6 10^11 vp	00C072	<10	77
		00C178	<10	26
		00C222	<10	423
		00D011	<10	98
		00D023	<10	<10
		00D031	<10	<10
2	Ad24ΔE1gagΔOrf6Ad5Orf6 10^10 vp	99C168	<10	<10
		99C170	<10	<10
		99C173	<10	<10
3	Ad24ΔE1gagΔE4Ad5Orf6 10^10 vp	99C154	<10	<10
		99C158	<10	<10
		99C177	<10	<10
4	MRKAd5-HIVgag 10^11 vp	00C018	34	1017
		00C034	14	423
		00C058	46	934
5	MRKAd5-HIVgag 10^10 vp	99C218	20	99
		99C227	40	767
		99D185	17	342

FIG. 19

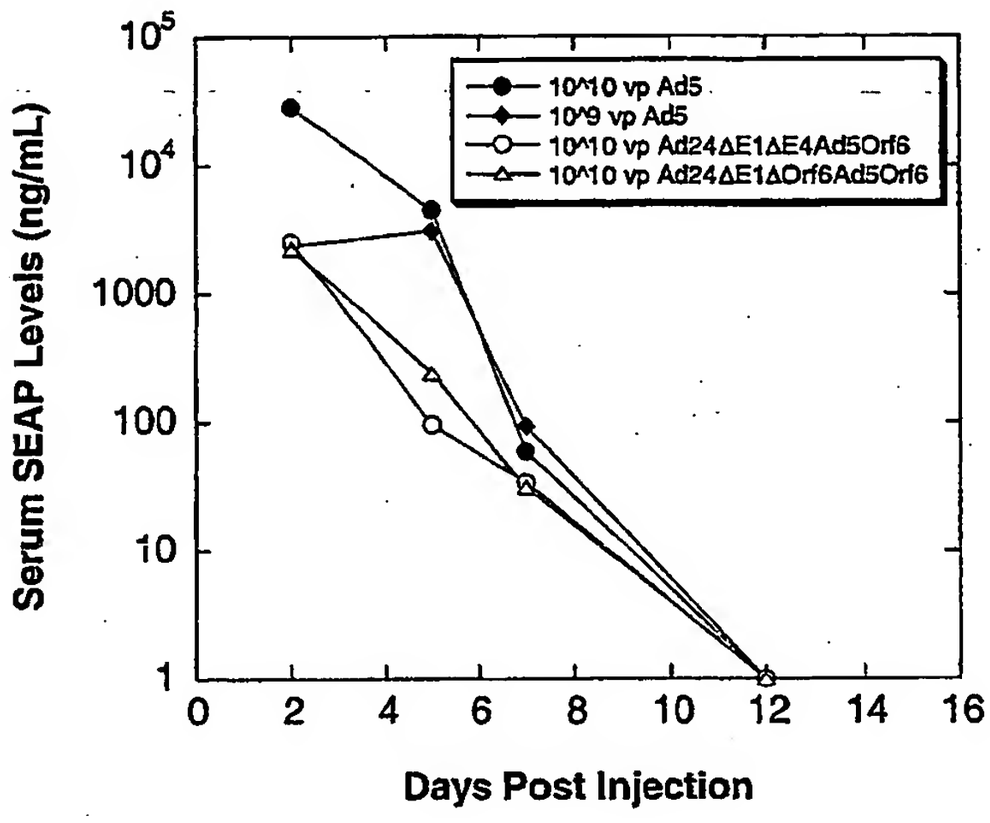

10^10 vp Ad5
10^9 vp Ad5
10^10 vp Ad24ΔE1ΔE4Ad5Orf6
10^10 vp Ad24ΔE1ΔOrf6Ad5Orf6
Serum SEAP Levels (ng/mL)
10^5
10^4
1000
100
10
1
0
2
4
6
8
10
12
14
16
Days Post Injection

FIG. 20

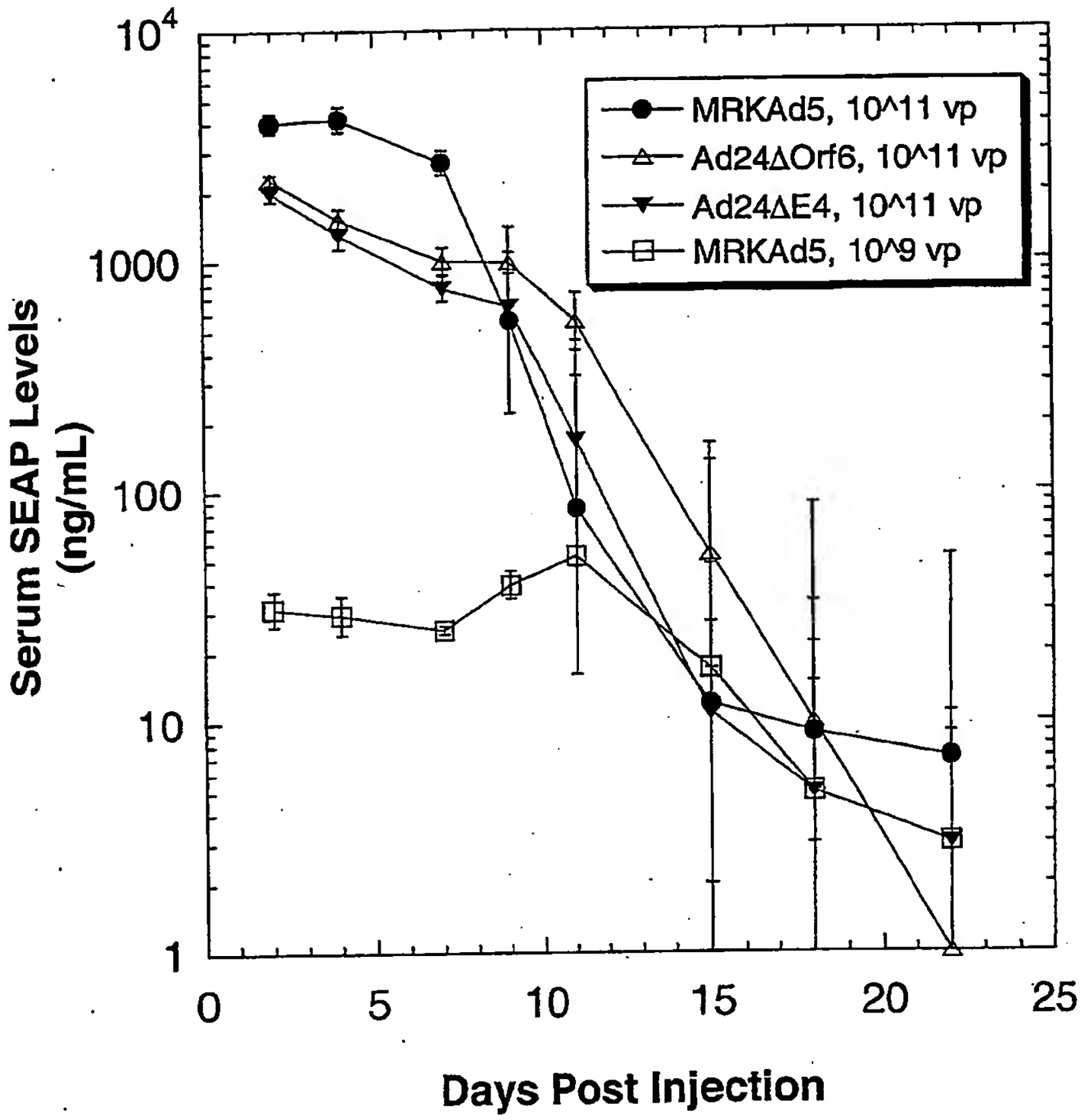

MRKAd5, 10^11 vp
Ad24ΔOrf6, 10^11 vp
Ad24ΔE4, 10^11 vp
MRKAd5, 10^9 vp
Serum SEAP Levels (ng/mL)
10^4
1000
100
10
1
0
5
10
15
20
25
Days Post Injection

FIG. 21

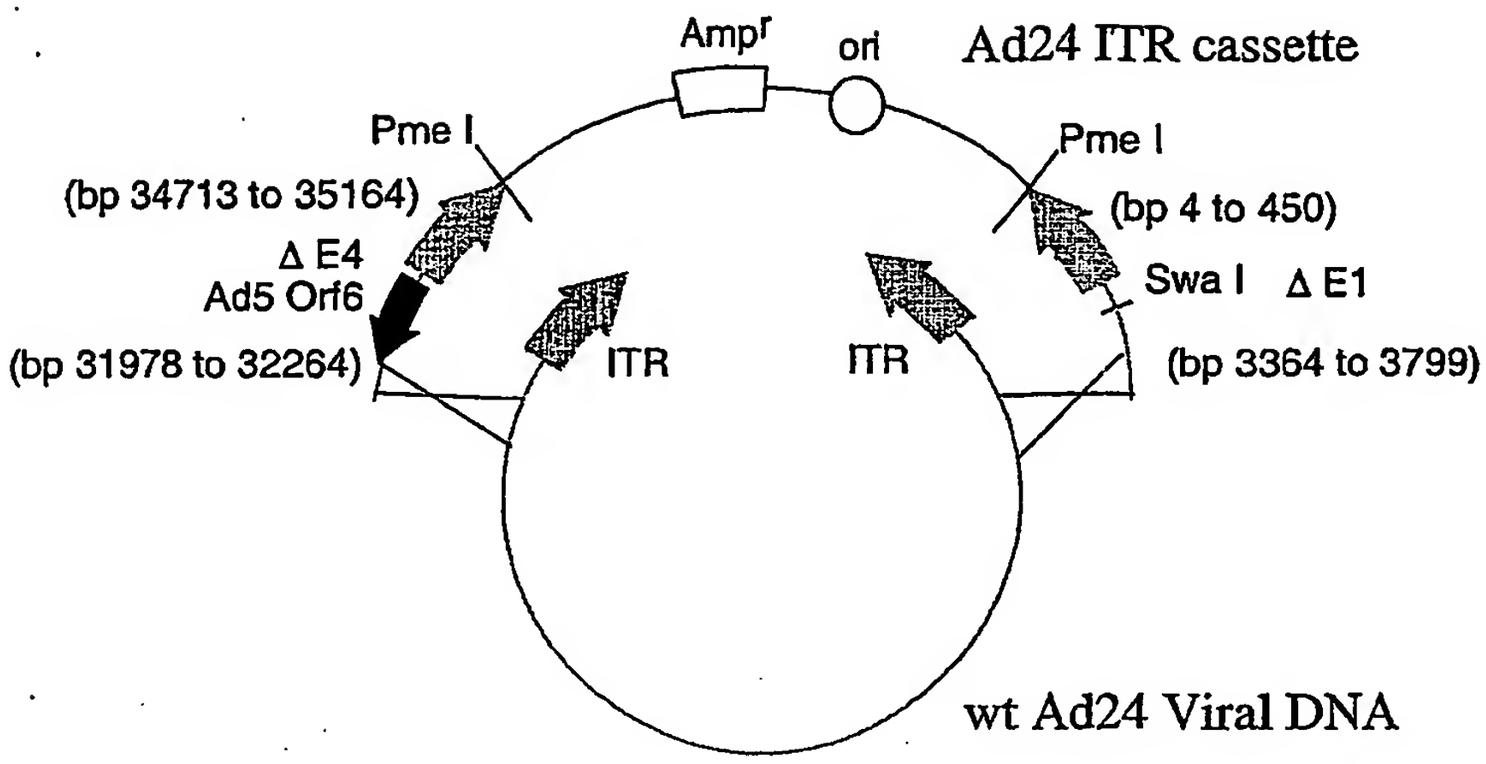

Ampr
ori
Ad24 ITR cassette
Pme I
(bp 34713 to 35164)
Δ E4
Ad5 Orf6
(bp 31978 to 32264)
ITR
ITR
Pme I
(bp 4 to 450)
Swa I Δ E1
(bp 3364 to 3799)
wt Ad24 Viral DNA

FIG. 22

Animal	Prime (Wk 0, 4, 26)	Boost (Wk 56)	Pre		Prime[b]		Pre-Boost[c]		Post-Boost[d]	
			Mock[a]	Gag[a]	Mock	Gag	Mock	Gag	Mock	Gag
Monkey 1	10^8 vp MRKAd5-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	18	16	1	244	3	74	3	1235
Monkey 2	10^7 vp MRKAd5-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	10	9	4	83	0	18	0	856
Monkey 3	10^8 vp MRKAd6-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	1	0	219	9	69	0	703
Monkey 4	10^7 vp MRKAd6-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	1	3	59	1	20	0	419
Monkey 5	none	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	3	4	ND[e]	ND	ND	ND	4	558
Monkey 6	none	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	0	3	ND	ND	ND	ND	1	295
Monkey 7	none	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	1	9	ND	ND	ND	ND	9	103
Monkey 8	none	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	3	3	ND	ND	ND	ND	1	381
Monkey 9	none	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	0	6	ND	ND	ND	ND	0	369
Monkey 10	none	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	15	5	ND	ND	ND	ND	10	211

FIG. 23

Animal	Prime (Wk 0, 4, 26)	Boost (Wk 56)	Gag-Specific T cells (Wk 60)	
			%CD4	%CD8
Monkey 1	10^9 vp MRKAd5-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	0.06	0.37
Monkey 2	10^7 vp MRKAd5-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	0.01	0.56
Monkey 3	10^9 vp MRKAd6-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	0.07	0.06
Monkey 4	10^7 vp MRKAd6-gag	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	0.04	0.20

FIG. 24

Animal	Prime (Wk 0, 4)	Boost (Wk 24)	Pre		Prime[b]		Pre-Boost[c]		Post-Boost[d]	
			Mock[a]	Gag[a]	Mock	Gag	Mock	Gag	Mock	Gag
Monkey 11	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	10^{7} vp MRKAd5-gag	3	4	3	150	4	28	0	188
Monkey 12	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	10^{7} vp MRKAd5-gag	0	3	1	753	4	554	0	1029
Monkey 13	10^{11} vp Ad24ΔE1gagΔOrf6Ad5Orf6	10^{7} vp MRKAd5-gag	1	9	4	273	0	370	0	1520
Monkey 14	none	10^{7} vp MRKAd5-gag	0	0	ND[e]	ND	ND	ND	4	94
Monkey 15	none	10^{7} vp MRKAd5-gag	0	0	ND	ND	ND	ND	1	168
Monkey 16	none	10^{7} vp MRKAd5-gag	8	9	ND	ND	ND	ND	8	149

FIG. 25

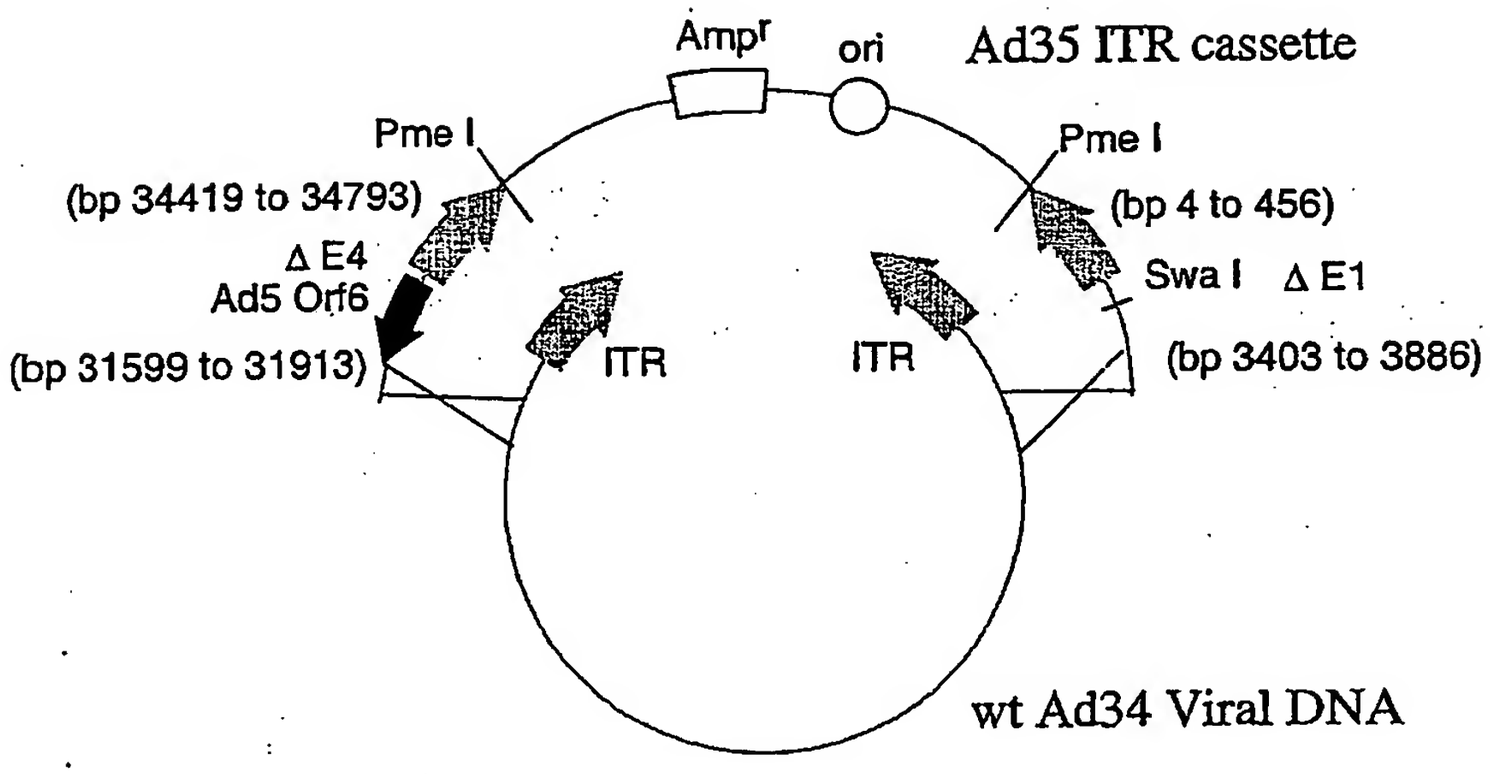

Amp^r
ori
Ad35 ITR cassette
Pme I
(bp 34419 to 34793)
Δ E4
Ad5 Orf6
(bp 31599 to 31913)
ITR
Pme I
(bp 4 to 456)
Swa I
Δ E1
(bp 3403 to 3886)
ITR
wt Ad34 Viral DNA

FIG. 26

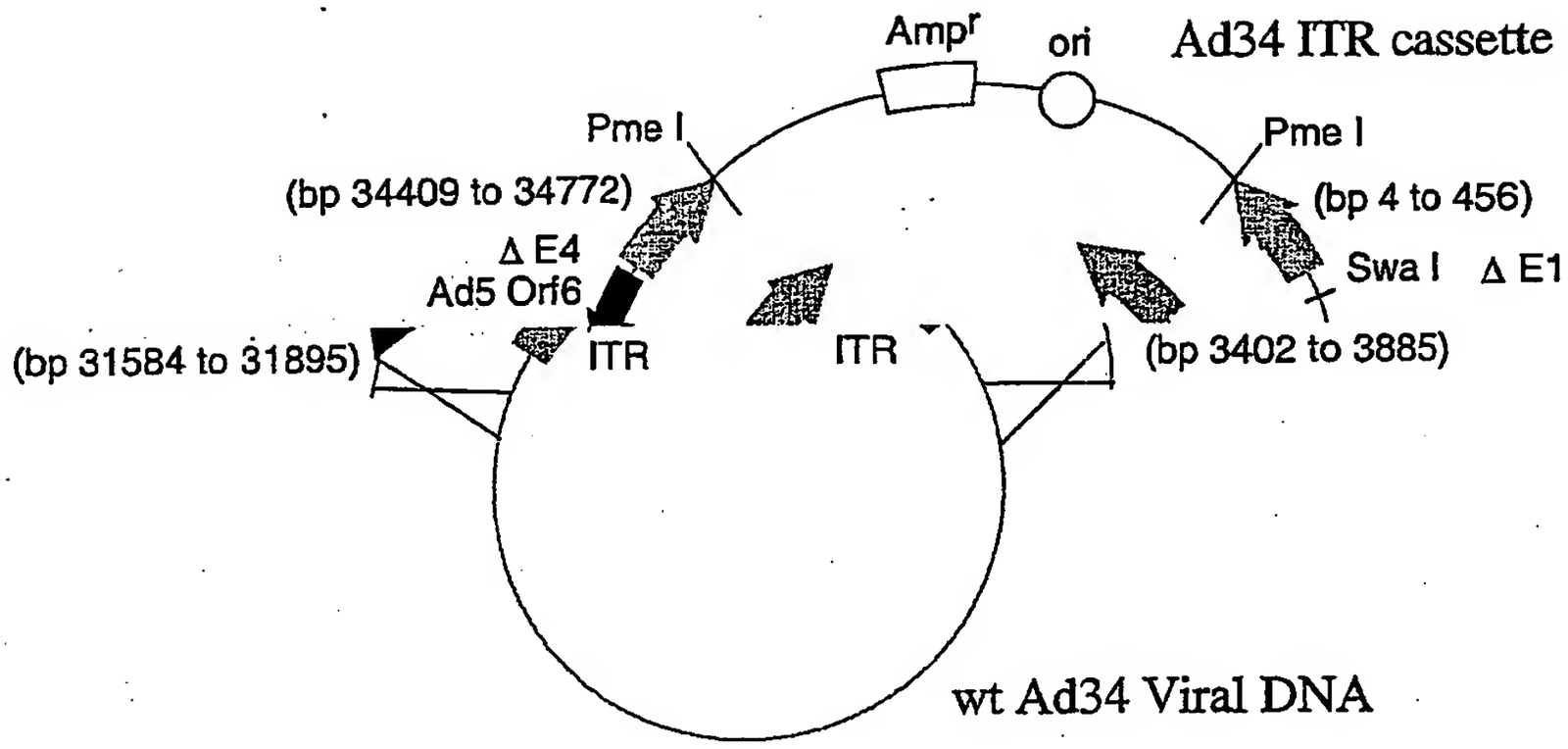

Amp^r
ori
Ad34 ITR cassette
Pme I
Pme I
(bp 34409 to 34772)
(bp 4 to 456)
Δ E4
Ad5 Orf6
Swa I
Δ E1
(bp 31584 to 31895)
ITR
ITR
(bp 3402 to 3885)
wt Ad34 Viral DNA

FIG. 27

```
   1 catcatcaat aatatacctt atagatggaa tggtgccaat atgtaaatga ggtgatttta
  61 aaaattgtgg ggtgtgtggt gattggctgt ggggttaacg gctaaacggg gcggcgcggc
 121 cgtgggaaaa tgacgttttg tgggggtgga gtttttttgc aagttgtcgc gggaaatgtg
 181 acgcataaaa aggctttttt tctcacggaa ctactgactt ttcccacggt atttaacagg
 241 aaatgaggta gttttgaccg gatgcaagtg aaaattgctg atttgcgcgc gaaaactgaa
 301 tgaggaagtg tttttctgaa taatgtggta tttatggcag ggtggagtat ttgttcaggg
 361 ccaggtagac tttgacccat tacgtggagg tttcgattac cgtgtttttt acctgaattt
 421 ccgcgtaccg tgtcaaagtc ttctgttttt acgtaggtgt cagctgatcg ctacggtatt
 481 tatacctcag ggtttgtgtc aagaggccac tcttgagtgc cagcgagaag agttttctcc
 541 tctgcgccgg cagtttaata ataaaaaaat gagagatttg cgatttctgc ctcaggaaat
 601 aatttctgct gagactggaa atgaaatact ggagcttgtg gtgcacgccc tgatgggaga
 661 cgatccggag ccacctgtgc agctttttga gcctcctacg cttcaggaac tgtatgattt
 721 agaggtagag ggatcggagg attctaatga ggaagctgtg aatggctttt ttaccgattc
 781 tatgctttta gctgctaatg aaggattaga attagatccg cctttggaca ctttcgatac
 841 tccaggggtg attgtggaaa gcggtacagg tgtaagaaaa ttacctgatt tgggttccgt
 901 ggactgtgat ttgcactgct atgaagacgg gtttcctccg agtgatgagg aggaccatga
 961 aaaggagcag tctatgcaga ctgcagcggg tgagggagtg aaggctgcca gtgttggttt
1021 tcagttggat tgcccggagc ttcctggaca tggctgtaag tcttgtgaat ttcacaggaa
1081 aaatactgga gtaaaggaac tgttatgttc gctttgttat atgagagcgc actgccactt
1141 tatttacagt aagtgtgttt aagttaaaat ttaaaggaat atgctgtttt tcacatgtat
1201 attgagtggg agttttgtgc ttcttattat aggtcctgtg tctgatgctg atgagtcacc
1261 atctcctgat tctactacct cacctcctga gattcaagca cctgttcctg tggacgtgcg
1321 caagcccatt cctgtgaagc ttaagcctgg gaaacgtcca gcagtggaaa aacttgagga
1381 cttgttacag ggtggggacg gacctttgga cttgagtaca cggaaacggc caagacaata
1441 agtgttccat atccgtgttt acttaaggtg acgtcaatat ttgtgtgaga gtgcaatgta
1501 ataaaaatat gttaactgtt cactggtttt tattgctttt tgggcgggga ctcaggtata
1561 taagtagaag cagacctgta tggttagctc ataggagctg gctttcatcc atggaggttt
1621 gggccatttt ggaagacctt agaaagacta ggcaactgtt agaggacgct tcggacggag
1681 tctccggttt ttggagattc tggttcgcta gtgaattagc tagggtagtt tttaggataa
1741 aacaggacta taaagaagaa tttgaaaagt tgttggtaga ttgcccagga ctttttgaag
1801 ctcttaattt gggccatcaa gttcacttta aagaaaaagt tttatcagtt ttagactttt
1861 caaccccagg tagaactgcc gctgctgtgg cttttcttac ttttatatta gataaatgga
1921 tcccgcagac tcatttcagc aggggatacg ttttggattt cgtagccaca gcattgtgga
1981 gaacatggaa ggttcgcaag atgaggacaa tcttaggtta ctggccagtg cagcctttgg
2041 gtgtagcggg aatcctgagg catccaccgg tcatgccagc ggttctggag gaggaacagc
2101 aagaggacaa cccgagagcc ggcctggacc ctccagtgga ggaggcggag tagctgactt
2161 gtctcctgaa ctgcaacggg tgcttactgg atctacgtcc actggacggg ataggggcgt
2221 taagagggag agggcatcta gtggtactga tgctagatct gagttggctt taagtttaat
2281 gagtcgcaga cgtcctgaaa ccatttggtg gcatgaggtc cagaaagagg gaagggatga
2341 agtttctgta ttgcaggaga aatattcact ggaacaggtg aaaacatgtt ggttggagcc
2401 tgaggatgat tgggaggtgg ccattaaaaa ttatgccaag atagctttga ggcctgataa
2461 acagtataag attactagac ggattaatat ccggaatgct tgttacatat ctggaaatgg
2521 ggctgaggtg gtaatagata ctcaagacaa ggcagttatt agatgctgca tgatggatat
2581 gtggcctgga gtagtcggta tggaagcagt aacttttgta aatgttaagt ttaggggaga
2641 tggttataat ggaatagtgt ttatggccaa taccaaactt atattgcatg gttgtagctt
2701 ttttggtttc aacaatacct gtgtagatgc ctggggacag gttagtgtac ggggatgtag
2761 tttctatgcg tgttggattg ccacagctgg cagaaccaag agtcaattgt ctctgaagaa
2821 atgcatattc caaagatgta acctgggcat tctgaatgaa ggcgaagcaa gggtccgcca
2881 ctgcgcttct acagatactg gatgttttat tttaattaag ggcaatgcca gcgtaaagca
2941 taacatgatt tgcggtgctt ccgatgagag gccttatcaa atgctcactt gtgccggtgg
3001 gcattgtaat atgctggcta ctgtgcatat tgtttcccat caacgcaaaa aatggcctgt
3061 ttttgatcac aatgtgttga ccaagtgtac catgcatgca ggtgggcgta gaggaatgtt
3121 tatgccttac cagtgtaaca tgaatcatgt gaaagtgttg ttggaaccag atgccttttc
3181 cagaatgagc ctaacaggaa tctttgacat gaacatgcaa atctggaaga tcctgaggta
3241 tgatgatacg agatcgaggg tgcgcgcatg cgaatgcgga ggcaagcatg ccaggttcca
3301 gccggtgtgt gtagatgtga ctgaagatct gagaccggat catttggtta ttgcccgcac
3361 tggagcagag ttcggatcca gtggagaaga aactgactaa ggtgagtatt gggaaaactt
3421 ggggtggggt tttcagatgg acagattgag taaaaatttg ttttttctgt ctttcagctg
3481 tcatgagtgg aaacgcttct tttaaggggg gagtcttcag cccttatctg acagggcgtc
3541 tcccatcctg ggcaggagtt cgtcagaatg ttatgggatc tactgtggat ggaagacccg
3601 tccaacccgc caattcttca acgctgacct atgctacttt aagttcttca cctttggacg
3661 cagctgcagc cgccgccgcc gcctctgttg ccgctaacac tgtgcttgga atgggttact
3721 atggaagtat cgtggctaat tccacttcct ctaataaccc ttctaccctg actcaggaca
3781 agttacttgt ccttttggcc cagctggagg ctttgaccca acgtctgggt gaactttatc
3841 agcaggtggc cgagttgcga gtacaaactg agtctgctgt cggcacggca aagtctaaat
```

FIG. 28A-1

3901 aaaaaaaaat tccacaatca atgaataaat aaacgagctt gttgttgatt taaaatcaag
3961 tgtttttatt tcatttttcg cgcacggtat gccctagacc accgatctcg atcattgaga
4021 acacggtgga ttttttccag aatcctatag aggtgggatt gaatgtttag atacatgggc
4081 attaggccat ctttggggtg gagatagctc cattgaaggg attcatgctc cggggtagtg
4141 ttgtaaatca cccagtcata acaaggtcgc agtgcatggt gttgcacaat atcttttaga
4201 agtaggctga ttgccacaga taagcccttg gtgtaggtgt ttacaaaccg gttgagctgg
4261 gaggggtgca ttcggggtga aattatgtgc attttggatt ggatttttaa gttggcaata
4321 ttgccgccaa gatctcgtct tgggttcatg ttatgaagga ccaccaagac ggtgtatccg
4381 gtacatttag gaaatttatc gtgtagcttg gatggaaaag cgtggaaaaa tttggagaca
4441 cccttgtgtc ctccgagatt ttccatgcac tcatccatga taatagcaat ggggccgtgg
4501 gcagcagcgc gggcaaacac gttccgtggg tctgacacat catagttatg ttcctgagtt
4561 aaatcatcat aagccatttt aatgaatttg gggcggagag tacccgattg gggtatgaat
4621 gttccttcgg gccccggagc atagttcccc tcacagattt gcatttccca agctttcagt
4681 tccgatggtg gaatcatgtc cacctggggg gctatgaaga acaccgtttc tggggcgggg
4741 gtgattagtt gggatgatag caagtttctg agcaattgag atttgccaca tccggtgggg
4801 ccataaatga ttccgattac aggttgcagg tggtagttta gggaacggca actgccgtct
4861 tctcgaagca agggggccac ctcgttcatc atttccctta catgcatatt ttcccgcacc
4921 aaatccatta ggaggcgctc tcctcctagt gatagaagtt cttgtagtga ggaaaagttt
4981 ttcagcggtt ttagaccgtc agccatgggc attttggaga gagtttgctg caaaagttct
5041 agtctgttcc acagttcagt gatgtgttct atggcatctc gatccagcag acctcctcgt
5101 ttcgcgggtt tggacggctc ctggagtagg gtatgagacg atgggcgtcc agcgctgcca
5161 gggttcggtc cttccagggt ctcagtgttc gagtcagggt tgtttccgtc acagtgaagg
5221 ggtgtgcgcc tgcttgggcg cttgccaggg tgcgcttcag actcattctg ctggtggaga
5281 acttctgtcg cttggcgccc tgtatgtcgg ccaagtagca gtttaccatg agttcgtagt
5341 tgagcgcctc ggctgcgtgg cctttggcgc ggagcttacc tttggaagtt ttcttgcata
5401 ccgggcagta taggcatttc agcgcataca gcttgggcgc aaggaaaatg gattctgggg
5461 agtatgcatc tgcgccgcag gaggcgcaaa cagtttcaca ttccaccagc caggttaaat
5521 ccggttcatt ggggtcaaaa acaagttttc cgccatattt tttgatgcgt ttcttacctt
5581 tggtctccat gagttcgtgt cctcgttgag tgacaaacag gctgtccgta tccccgtaga
5641 ctgattttac aggcctcttc tccagtggag tgcctcggtc ttcttcgtac aggaactctg
5701 accactctga tacaaaggcg cgcgtccagg ccagcacaaa ggaggctatg tgggaggggt
5761 agcgatcgtt gtcaaccagg gggtccacct tttccaaagt atgcaaacac atgtcaccct
5821 cttcaacatc caggaatgtg attggcttgt aggtgtattt cacgtgacct ggggtccccg
5881 ctgggggggt ataaaagggg gcggttcttt gctcttcctc actgtcttcc ggatcgctgt
5941 ccaggaacgt cagctgttgg ggtaggtatt ccctctcgaa ggcgggcatg acctctgcac
6001 tcaggttgtc agtttctaag aacgaggagg atttgatatt gacagtgccg gttgagatgc
6061 ctttcatgag gttttcgtcc atttggtcag aaaacacaat ttttttattg tcaagtttgg
6121 tggcaaatga tccatacagg gcgttggata aaagtttggc aatggatcgc atggtttggt
6181 tcttttcctt gtccgcgcgc tctttggcag cgatgttgag ttggacatac tcgcgtgcta
6241 ggcacttcca ttcggggaag atagttgtca attcatctgg cacgattctc acttgccacc
6301 ctcgattatg caaggtaatt aaatccacac tggtggccac ctcgcctcga aggggttcgt
6361 tggtccaaca gagcctacct cctttcctag aacagaaagg gggaagtggg tctagcataa
6421 gttcatcggg agggtctgca tccatggtaa agattcccgg aagtaaatcc ttatcaaaat
6481 agctgatggg agtggggtca tctaaggcca tttgccattc tcgagctgcc agtgcacgct
6541 catatgggtt aaggggactg ccccagggca tgggatgggt gagtgcagag gcatacatgc
6601 cacagatgtc atagacgtag atgggatcct caaagatgcc tatataggtt ggatagcatc
6661 gccccccctct gatacttgct cgcacatagt catatagttc atgtgatggc gctagcaacc
6721 ccggacccaa gttggtgcga ttgggttttt ctgttctgta gacaatctgg cgaaagatgg
6781 cgtgagaatt ggaagagatg gtgggtcttt gaaaaatgtt gaaatgggca tgaggtagac
6841 ctacagagtc tctgacaaag tgggcataag attcttgaag cttggttacc agttcggcgg
6901 tgacaagtac gtctagggcg cagtagtcaa gtgtttcttg aatgatgtca taacctggtt
6961 ggtttttctt ttcccacagt tcgcggttga gaaggtattc ttcgcgatcc ttccagtact
7021 cttctagcgg aaacccgtct ttgtctgcac ggtaagatcc tagcatgtag aactgattaa
7081 ctgccttgta agggcagcag cccttctcta cgggtagaga gtatgcttga gcagcttttc
7141 gcagcgaagc gtgagtaagg gcgaaggtgt ctctgaccat gactttgaga aattggtatt
7201 tgaagtccat gtcgtcacag gctccctgtt cccagagttg gaagtctacc cgtttcttgt
7261 aggcggggtt gggcaaagcg aaagtaacat cgttgaagag aatcttaccg gctctgggca
7321 taaaattgcg agtgatgcgg aaaggctgtg gtacttccgc tcgattgttg atcacctggg
7381 cagctaggac gatctcgtcg aaaccgttga tgttgtgtcc tacgatgtat aattctatga
7441 aacgcggcgt gcctttgacg tgaggtagct tattgagctc atcaaaggtt aggtctgtag
7501 ggtcagataa ggcgtagtgt tcgagagccc attcgtgcag gtgaggattt gcatgtagga
7561 atgatgacca aagatccacc gccagtgctg tttgtaactg gtcccgatac tgacgaaaat
7621 gctggccaat tgccattttt tctggagtga cacagtagaa ggttctgggg tcttgttgcc
7681 atcgatccca ctttagttta atggctagat cgtgggccat gttgacgaga cgctcttctc
7741 ctgagagttt catgaccagc atgaaaggaa ctagttgttt gccaaaggac cccatccagg

FIG. 28A-2

```
7801 tgtaagtttc cacatcgtag gtcaggaaga gtctttctgt gcgaggatga gagccgatcg
7861 ggaagaactg gatttcctgc caccagttgg aggattggct gttgatgtga tggaagtaga
7921 agtttctgcg gcgcgccgag cattcgtgtt tgtgcttgta cagacggccg cagtagtcgc
7981 agcgttgcac gggttgtatc tcgtgaatga gctgtacctg gcttcccttg acgagaaatt
8041 tcagtgggaa gccgaggcct ggcgattgta tctcgtgctc ttctatattc gctgtatcgg
8101 cctgttcatc ttctgtttcg gtggtggtca tgctgacgag cccccgcggg aggcaagtcc
8161 agacctcggc gcgggagggg cggagctgaa ggaccagagc gcgcaggctg gagctgtcca
8221 gagtcctgag acgctgcgga ctcaggttag taggtaggga cagaagatta acttgcatga
8281 tcttttccag ggcgtgcggg aggttcagat ggtacttgat ttccacaggt tcgtttgtag
8341 agatgtcaat ggcttgcagg gttccgtgtc ctttgggcgc cactaccgta cctttgtttt
8401 ttcttttgat cggtggtggc tctcttgctt cttgcatgct cagaagcgat gacggggacg
8461 cgcgccgggc ggaagcggtt gttccggacc cggaggcatg gctggtagtg gcacgtcggc
8521 gccgcgcacg ggcaggttct ggtactgcgc tctgagaaga cttgcgtgcg ccaccacgcg
8581 tcgattgacg tcttgtatct gacgtctctg ggtgaaagct accggccccg tgagcttgaa
8641 cctgaaagag agttcaacag aatcaatttc ggtatcgtta acggcagctt gtctcagtat
8701 ttcttgtacg tcaccagagt tgtcctggta ggcgatctcc gccatgaact gctcgatttc
8761 ttcctcctga agatctccgc gacccgctct ctcgacggtg gccgcgaggt cattggagat
8821 acggcccatg agttgggaga atgcagtcat gcccgcctcg ttccagacgc ggctgtaaac
8881 cacggccccc tcggagtctc ttgcgcgcat caccacctga gcgaggttaa gctccacgtg
8941 tctggtgaag accgcatagt tgcataggcg ctgaaaaagg tagttgagtg tggtggcaat
9001 gtgttcggcg acgaagaaat acatgatcca tcgtctcagc ggcatttcgc tgacatcgcc
9061 cagagcttcc aagcgctcca tggcctcgta gaagtccacg gcaaaattaa aaaactggga
9121 gtttcgcgcg gacacggtca attcctcctc gagaagacgg atgagttcgg ctatggtggc
9181 ccgtacttcg cgttcgaagg ctcccgggat ctcttcttcc tcttctatct cttcttccac
9241 taacatctct tcttcgtctt caggcggggg cggaggggcc acacggcgac gtcgacggcg
9301 cacgggcaaa cggtcgatga atcgttcaat gacctctccg cggcggcggc gcatggtttc
9361 agtgacggcg cggccgttct cgcgcggtcg cagagtaaaa acaccgccgc gcatctcctt
9421 aaagtggtga ctgggaggtt ctccgtttgg gagggagagg gcgctgatta tacattttat
9481 taattggccc gtagggactg cgcgcagaga tctgatcgtg tcaagatcca cgggatctga
9541 aaacctttcg acgaaagcgt ctaaccagtc acagtcacaa ggtaggctga gtacggcttc
9601 ttgtgggcgg gggtggttat gtgttcggtc tgggtcttct gtttcttctt catctcggga
9661 aggtgagacg atgctgctgg tgatgaaatt aaagtaggca gttctaagac ggcggatggt
9721 ggcgaggagc accaggtctt tgggtccggc ttgctggata cgcaggcgat tggccattcc
9781 ccaagcatta tcctgacatc tagcaagatc tttgtagtag tcttgcatga gccgttctac
9841 gggcacttct tcctcacccg ttctgccatg catacgtgtg agtccaaacc cgcgcattgg
9901 ttgtaccagt gccaagtcag ctacgactct ttcggcgagg atggcttgct gtacttgggt
9961 gagggtggct tgaaagtcat caaaatccac aaagcggtgg taagccccgg tattaatggt
10021 gtaagcacag ttggccatga ctgaccagtt aactgtctgg tgaccagggc gcacgagctc
10081 ggtgtattta aggcgcgaat aggcgcgggt gtcaaagatg taatcgttgc aggtgcgcac
10141 cagatactgg taacctataa gaaaatgcgg cggtggttgg cggtagagag gccatcgttc
10201 tgtagctgga gcgccggggg cgaggtcttc caacataagg cggtgatagc cgtagatgta
10261 cctggacatc caggtgattc ctgcggcggt agtagaagcc cgaggaaact cgcgtacgcg
10321 gttccaaatg ttgcgtagcg gcatgaagta gttcattgta ggcacggttt gaccagtgag
10381 gcgcgcgcag tcattgatgc tctatagaca cggagaaaat gaaagcgttc agcgactcga
10441 ctccgtagcc tggaggaacg tgaacgggtt gggtcgcggt gtaccccggt tcgagacttg
10501 tactcgagcc ggccggagcc gcggctaacg tggtattggc actcccgtct cgacccagcc
10561 tacaaaaatc caggatacgg aatcgagtcg ttttgctggt tgccgaatgg cagggaagtg
10621 agtcctattt ttttttttg ccgctcagat gcatcccgtg ctgcgacaga tgcgtcccca
10681 acaacagccc ccctcgcagc agcagcaacc acaaaaggct gtccctgcaa ctactgcaac
10741 tgccgctgtg agcggtgcgg gacagcccgc ctatgatctg gacttggaag agggcgaagg
10801 actggcacgt ctaggtgcgc cttcgcccga gcggcatccg cgagttcaac tgaaaaaaga
10861 ttctcgcgag gcgtatgtgc cccaacagaa cctatttaga gacagaagcg gcgaggagcc
10921 ggaggagatg cgagcttccc gctttaacgc gggtcgtgag ctgcgtcacg gtttggacag
10981 aagacgagtg ttgcgggacg aggatttcga agttgatgaa gtgacaggga tcagtcctgc
11041 cagggcacac gtggctgcag ccaaccttgt atcggcttac gaacagacag taaaggaaga
11101 gcgtaatttc caaaagtctt ttaataatca tgtgcgaacc ctcattgccc gcgaagaagt
11161 caccttggt ttgatgcatt tgtgggattt gatggaagct atcattcaga accctactag
11221 caaacctctg accgcacagc tgtttctggt ggtgcaacac agcagagaca atgaggcttt
11281 cagagaggcg ctgctcaaca tcaccgaacc cgaggggaga tggttgtatg atcttatcaa
11341 cattctacag agtatcatag tgcaggagcg gagcctgggc ctggccgaga aggtggctgc
11401 catcaattac tcggttttga gcttgggaaa gtattacgct cgcaagatct acaagactcc
11461 atacgttccc atagacaagg aggtgaagat agatgggttc tacatgcgca tgacgctgaa
11521 ggtgttgacc ctgagcgatg atcttggggt gtaccgcaat gacagaatgc atcgcgcggt
11581 gagcgccagc aggaggcgcg agttaagcga cagggaactg atgcacagtt tgcaaagagc
11641 tctaactgga gctggaaccg agggtgagaa ttactttgat atgggagctg acttgcagtg
```

FIG. 28A-3

```
11701 gcagcctagt cgcagggctc tgaacgccgc gacggcagga tgtgagcttc cttacataga
11761 agaggcggat gaaggcgagg aggaagaggg cgagtacttg gaagactgat ggcacaaccc
11821 gtgtttttg ctagatggaa cagcaagcac cggatcccgc aatgcgggcg gcgctgcaga
11881 gccagccgtc cggcattaac tcctcggacg attggaccca ggccatgcaa cgtatcatgg
11941 cgttgacgac tcgcaacccc gaagccttta gacagcaacc ccaggccaac cgtctatcgg
12001 ccatcatgga agctgtagtg ccttcccgct ctaatcccac tcatgagaag gtcctggcca
12061 tcgtgaacgc gttggtggag aacaaagcta ttcgtccaga tgaggccgga ctggtataca
12121 acgctctctt agaacgcgtg gctcgctaca acagtagcaa tgtgcaaacc aatttggacc
12181 gtatgataac agatgtacgc gaagccgtgt ctcagcgcga aaggttccag cgcgatgcca
12241 acctgggttc gctggtggcg ttaaatgctt tcttgagtac tcagcctgct aatgtgccgc
12301 gtggtcaaca ggattatact aacttttaa gtgctttgag actgatggta tcagaagtac
12361 ctcagagcga agtatatcag tccggtcctg attacttctt tcagactagc agacagggct
12421 tgcagacggt aaatctgagc caagctttta aaaaccttaa aggtttgtgg ggagtgcatg
12481 ccccggtagg agaaagagca accgtgtcta gcttgttaac tccgaactcc cgcctattat
12541 tactgttggt agctcctttc accgacagcg gtagcatcga ccgtaattcc tatttgggtt
12601 acctactaaa cctgtatcgc gaagccatag ggcaaagtca ggtggacgag cagacctatc
12661 aagaaattac ccaagtcagt cgcgctttgg gacaggaaga cactggcagt ttggaagcca
12721 ctctgaactt cttgcttacc aatcggtctc aaaagatccc tcctcaatat gctcttactg
12781 cggaggagga gaggatcctt agatatgtgc agcagagcgt gggattgttt ctgatgcaag
12841 agggggcaac tccgactgca gcactggaca tgacagcgcg aaatatggag cccagcatgt
12901 atgccagtaa ccgacctttc attaacaaac tgctggacta cttgcacaga gctgccgcta
12961 tgaactctga ttatttcacc aatgccatct taaacccgca ctggctgccc ccacctggtt
13021 tctacacggg cgaatatgac atgcccgacc ctaatgacgg atttctgtgg gacgacgtgg
13081 acagcgatgt tttttcacct ctttctgatc atcgcacgtg gaaaaaggaa ggcggcgata
13141 gaatgcattc ttctgcatcg ctgtccgggg tcattggtgc taccgcggct gagcccgagt
13201 ctgcaagtcc ttttcctagt ctacccttt ctctacacag tgtacgtagc agcgaagtgg
13261 gtagaataag tcgcccgagt ttaatgggcg aagaggagta cctaaacgat tccttgctca
13321 gaccggcaag agaaaaaaat ttcccaaaca atggaataga aagtttggtg gataaaatga
13381 gtagatggaa gacttatgct caggatcaca gagacgagcc tgggatcatg gggactacaa
13441 gtagagcgag ccgtagacgc cagcgccatg acagacagag gggtcttgtg tgggacgatg
13501 aggattcggc cgatgatagc agcgtattgg acttgggtgg gagaggaagg ggcaacccgt
13561 ttgctcattt gcgccctcgc ttgggtggta tgttgtaaaa aaaaataaaa aagaaaaaac
13621 tcaccaaggc catggcgacg agcgtacgtt cgttcttctt tattatctgt gtctagtata
13681 atgaggcgag tcgtgctagg cggagcggtg gtgtatccgg agggtcctcc tccttcgtac
13741 gagagcgtga tgcagcagca gcaggcgacg gcggtgatgc aatccccact ggaggctccc
13801 tttgtgcctc cgcgatacct ggcacctacg gagggcagaa acagcattcg ttactcggaa
13861 ctggcacctc agtacgatac caccaggttg tatctggtgg acaacaagtc ggcggacatt
13921 gcttctctga actatcagaa tgaccacagc aacttcttga ccacggtggt gcaaaacaat
13981 gactttaccc ctacggaagc cagcacccag accattaact ttgatgaacg atcgcggtgg
14041 ggcggtcagc taaaaaccat catgcatact aacatgccca acgtgaacga gtatatgttt
14101 agtaacaagt tcaaagcgcg tgtgatggtg tccagaaaac ctcctgaggg tgttagagta
14161 gacgataatt atgatcataa gcaagatatt ctaaaatacg agtggttcga gtttactttg
14221 ccagaaggca acttttcggt cactatgact atcgacttga tgaacaatgc catcatagac
14281 aattacttga aagtgggcag acagaatgga gtgttggaaa gtgacattgg tgttaagttc
14341 gacactagga acttcaagtt gggatgggat ccagaaacta agttgatcat gcctggggtt
14401 tacacctatg aggccttcca tcctgacatc gtattgctgc ctggctgcgg agtggacttt
14461 accgaaagcc gtctgagcaa ccttcttggc attagaaaga aacacccatt ccaagagggt
14521 tttaagatct tgtatgagga tttagaagga ggaaatattc cagccctttt ggatgtagat
14581 gcttatgaga acagcaagaa agatcaaaaa gccaaaatag aagctgctgc agaagctaaa
14641 gcaaacatag ttgccaacga tccggtaagg gtggctaacg ctagtgaaat caggggagac
14701 agttttgccg caacatccgt tccgactaaa gaatcattat tggatgatgt gtctcaaaac
14761 atagagttaa aactcactat taagcctgtg gaaaaagatg gcaaaaacag aagttacaat
14821 gtgttggaag ataaaatcaa cacggcctat cgcagttggt acctttcgta caattatggc
14881 gaccccgaaa aaggagtgcg ttcctggaca ttgctcacca cctcagatgt cacctgcgga
14941 gcggagcagg tctactggtc gcttccagac atgatgcagg atcctgtcac tttccgctcc
15001 actagacaag tcagtaacta ccctgtggtg ggtgcagagc ttatgcccgt cttttcaaag
15061 agcttctaca acgaacaagc tgtgtactcc cagcagctcc gccagtccac ctcgcttacg
15121 cacgtcttca accgctttcc tgagaaccag attttaatcc gtccgccggc gcccacaatt
15181 accaccgtca gtgaaaacgt tcctgctctc acagatcacg ggaccctgcc gttgcgcagc
15241 agtatccggg gagtccaacg tgtgaccgtt actgacgcca gacgccgcac ctgtccctac
15301 gtgtacaagg cactgggcat agtcgcaccg cgcgtccttt caagccgcac tttctaaaaa
15361 aaaaaaaaaa atgtccgttc ttatctcgcc cagtaataac accggttggg gtctgcgcgc
15421 tcccagcaag atgtacggag gcgcacgcaa acgttctacc caacatcccg tgcgtgttcg
15481 cgggcatttt cgcgctccat ggggtgccct caagggccgc actcgcgttc gaaccaccgt
15541 cgatgatgta atcgatcagg tggttgccga cgcccgtaat tatactccta ctgcgcctac
```

FIG. 28A-4

```
15601 atctactgtg gacgcagtta ttgacagtgt agtggctgac gctcgcaact atgctcgacg
15661 taagagccgg cgaaggcgca ttgccagacg tcaccgagct accactgcca tgcgagcagc
15721 aagagctctg ctacgaagag ctagacgcgt ggggcgaaga gccatgctta gggcggccag
15781 acgtgcagct tcgggcgcca gcgccggcag gtcccgcagg caagcagccg ctgtcgcagc
15841 ggcgactatt gccgacatgg cccaatcgcg aagaggcaat gtatactggg tgcgtgacgc
15901 tgccaccggt caacgtgtac ccgtgcgcac ccgtcccgct cgcacttaga agatactgag
15961 cagtctccga tgttgtgtcc cagcggcgag gatgtccaag cgcaaataca aggaagaaat
16021 gctgcaggtt atcgcacctg aagtctacgg ccaaccgttg aaggatgaaa aaaaaccccg
16081 caaaatcaag cgggtaaaaa aggacaaaaa agaagaggaa gatggcgatg atgggctggc
16141 ggagtttgtg cgcgagtttg ccccacggcg acgcgtgcaa tggcgtgggc gcaaagttcg
16201 acatgtgttg agacctggaa cttcggtggt ctttacaccc ggcgagcgtt caagcgctac
16261 ttttaagcgt tcctatgatg aggtgtacgg ggatgatgat attcttgagc aggcagctga
16321 ccgattaggc gagtttgctt atggcaagcg tagtagaata aatcccaagg atgaaacagt
16381 gtccataccc ttggatcatg gaaatcccac ccctagtctt aaaccggtca ctttgcagca
16441 agtgttaccc gtaactccgc gaacaggtgt taaacgcgaa ggtgaagatt tgtatcccac
16501 tatgcaactg atggtgccca aacgccagaa gttggaggac gttttggaga aagtaaaagt
16561 ggatccagat attcaacctg aggttaaagt gagacccatt aagcaggtag cgcctggtct
16621 gggagtacaa actgtagaca ttaaaattcc cactgaaagt atggaagtgc aaactgaacc
16681 cgcaaagcct actgccacct ccactgaagt gcaaacggac ccatggatgc ccatgcctat
16741 tacaactgac gccgtcggtc ccactcgaag atcccgacga aagtacggtc cagcaagtct
16801 gttgatgccc aactatgtcg tacacccatc tattattcct actcctggtt accgaggcac
16861 tcgctactat cgcagccgaa acagtacttc ccgccgtcgc cgcaagacac ctgcaaatcg
16921 cagtcgtcgc cgtagacgca caagcaaacc gattcccggc gccctggtgc ggcaagtgta
16981 ccgcaatggt agtgcggaac ctttgacact gccgcgtgcg cgttaccatc ctagtatcat
17041 cacttaatca atgttgccgc tgcctccttg cagatatggc cctcacttgt cgccttcgcg
17101 ttcccatcac tggttaccga ggaagaaact cgcgccgtag aagagggatg ttggggcgcg
17161 gaatgcgacg ctacaggcga cggcgtgcta tccgcaagca attgcggggt ggtttttttgc
17221 cagccttaat tccaattatc gctgctgcga ttggcgcaat accaggcata gcttccgtgg
17281 cggttcaggc ctcgcaacga cattgacatt ggaaaaaaaa aaaacgtata aataaaaaat
17341 acaatggact ctgacactcc tggtactgtg actatgtttt cttagagatg gaagacatca
17401 atttttcatc cttggctccg cgacacggca cgaagccgta catgggcacc tggagcgaca
17461 tcggcacgag ccaactgaac gggggcgcct tcaattggag cagtatctgg agcgggctta
17521 aaaattttgg ctcaaccata aaaacatacg ggaacaaagc ttggaacagc agtacaggac
17581 aggcgcttag aaataaactt aaagaccaga acttccaaca aaaagtagtc gatgggatag
17641 cttccggtat caatggagtg gtagatttgg ctaaccaggc tgtgcagaaa aagataaaca
17701 gtcgtttgga cccgccgcca gcaaccccag gtgaaatgca agtggaggaa gaaattcctc
17761 cgccagaaaa acgaggcgac aagcgtccgc gtcccgattt ggaagagacg ctggtgacgc
17821 gcgtagatga accgccttct tatgaggaag caacgaagct tggaatgccc accactagac
17881 cgatagcccc tatggccacc ggggtgatga aaccttctca gttgcatcga cccgtcacct
17941 tggatttgcc ccctcctcct gctgctactg ctgtacccgc ttctaagcct gtcgctgccc
18001 cgaaaccagt cgccgtagcc aggtcacgtc ccgggggcgc tcctcgtcca aatgcacact
18061 ggcaaaatac tctgaacagc atcgtgggtc taggcgtgca aagtgtaaaa cgccgtcgct
18121 gcttttaatt aaatatggag tagcgcttaa cttgcctatc tgtgtatatg tgtcattaca
18181 cgccgtcaca gcatcagagg aaaaaaggaa gaggtcgtgc gtcgacgctg agttactttc
18241 aagatggcca ccccatcgat gctgccccaa tgggcataca tgcacatcgc cggacaggat
18301 gcttcggagt acctgagtcc gggtctggtg cagttcgccc gcgccacaga cacctacttc
18361 aatctgggaa ataagtttag aaatcctacc gtagcgccga cccacgatgt gaccaccgat
18421 cgtagccagc ggctcatgtt gcgcttcgtg cccgttgacc gggaggacaa tacatactct
18481 tacaaagtgc ggtacaccct ggccgtgggc gacaacagag tgctggatat ggccagcacg
18541 ttctttgaca ttaggggcgt gttggacaga ggtcccagtt ttaaacccta ttctggtacg
18601 gcttacaact ccctggctcc taaaggcgct ccaaatgcat ctcagtggtt ggataaggga
18661 gttacaagca ctggcctagt ggacgacggc aatactgatg atggggaaga agccaaaaaa
18721 gcaacataca cttttggtaa tgctccagta aaagccgagg ctgaaatcac aaaagacgga
18781 ttgccggtgg gcttggaagt ttcaactgaa ggtcctaaac caatctatgc tgataagctt
18841 tatcagccag aacctcaagt gggagacgaa acttggactg acctagacgg aaaaaccgaa
18901 gagtatggag ggagggttct taaacctgaa actaaaatga aaccctgcta cggatctttt
18961 gctaaaccta ctaatattaa aggaggtcag gcaaaggtaa aaccaaaaga agacgatggc
19021 actaacaaca tcgagtatga cattgacatg aacttctttg acttaagatc acaaagatca
19081 gaactcaaac ctaaaattgt aatgtatgca gaaaatgtgg acctggaatg tccagatact
19141 catgttgtgt acaaacctgg agtttcagat gctagttctg agaccaatct tggacaacag
19201 tctatgccca acagacccaa ctacattggc ttcagagata acttcatcgg acttatgtac
19261 tataacagta ctggcaacat gggggtactg gctggccaag cgtctcagtt gaatgcagtg
19321 gttgacttgc aggacagaaa cacagaactg tcttaccaac tcttgcttga ctctctgggc
19381 gacagaacca gatactttag catgtggaat caggctgtgg acagttatga tcctgatgta
19441 cgtgttattg aaaatcatgg tgtggaagat gaacttccca actattgttt tccgttggat
```

FIG. 28A-5

```
19501 ggtgtcggtc cgcgaacaga tagttacaag gagattaagc caaatggaga ccaatctact
19561 tggacaaatg tagacccaac tggcagcagt gaacttgcta agggaaatcc atttgccatg
19621 gaaattaacc ttcaagccaa tctatggcga agtttccttt attccaatgt ggctctatat
19681 ctcccagact cgtacaaata caccccgtcc aatgtcactc ttccagaaaa caaaaacacc
19741 tacgactaca tgaacgggcg ggtggtgccg ccatctctag tagacaccta tgtgaacatt
19801 ggtgccaggt ggtctctgga tgccatggac aatgtcaacc cattcaacca ccaccgtaac
19861 gctggcttgc gttaccgatc catgcttctg ggtaacggac gttatgtgcc tttccacata
19921 caagtgcctc aaaaattctt cgctgttaaa aacctgctgc ttctcccagg ctcctacact
19981 tatgagtgga actttaggaa ggatgtaaac atggttctac agagttccct cggtaacgac
20041 ctacgggtag atggcgccag catcagtttt acgagcatca acctctatgc tacttttttc
20101 cccatggctc acaacaccgc ttccaccctt gaagccatgc tgcggaatga caccaatgat
20161 cagtcattca acgactacct atctgcagct aacatgctct accccattcc tgccaatgca
20221 accaatattc ccatttccat tccttctcgc aactgggcgg ctttcagagg ctggtcattt
20281 accagactga aaaccaaaga aactccctct ttggggtctg gatttgaccc ctacttcgtc
20341 tattctggtt ctattcccta cctggatggt accttctacc tgaaccacac ttttaagaag
20401 gtttccatca tgtttgactc ttcagtgagc tggcctggaa atgacaggtt actatctcct
20461 aacgaatttg aaataaagcg cactgtggat ggcgaaggct acaacgtagc ccaatgcaac
20521 atgaccaaag actggttctt ggtacagatg ctcgccaact acaacatcgg ctatcagggc
20581 ttctacattc cagaaggata caaagatcgc atgtattcat ttttcagaaa cttccagccc
20641 atgagcaggc aggtggttga tgaggtcaat tacaaagact tcaaggccgt cgccataccc
20701 taccaacaca acaactctgg ctttgtgggt tacatggctc cgaccatgcg tcaaggtcaa
20761 ccctatcccg ctaactatcc ctatccactc attggaacaa ctgccgtaaa tagtgttacg
20821 cagaaaaagt tcttgtgtga cagaaccatg tggcgcatac cgttctcaag caacttcatg
20881 tctatgggag cccttacaga cttgggacag aacatgctct atgccaactc agctcatgct
20941 ctggacatga cctttgaggt ggatcccatg gatgagccca ccctgcttta tcttctcttc
21001 gaagttttcg acgtggtcag agtgcatcag ccacaccgcg gcatcatcga ggcagtctac
21061 ctgcgtacac cgttctcggc cggtaacgct accacgtaag aagcttcttg cttcttgcaa
21121 acagcagctg caaccatggc ctgcggatcc caaaacggct ccagcgagca agagctcaga
21181 gccattgtcc aagacctggg ttgcggacca tatttttgg gaacctttga taagcgcttc
21241 ccggggttca tggcccccga taagctcgcc tgtgccattg taaatacggc cggacgtgag
21301 acgggggggag agcactggtt ggctttcggt tggaacccac gttctaacac ctgctacctt
21361 tttgatcctt ttggattctc ggatgatcgt ctcaaacaga tttaccagtt tgaatatgag
21421 ggtctcctgc gccgcagcgc tcttgctacc aaggaccggt gtattacgct ggaaaaatct
21481 acccagaccg tgcagggccc ccgttctgcc gcctgcggac ttttctgctg catgttcctt
21541 catgcctttg tgcactggcc tgaccgtccc atggacggaa accccaccat gaaattgcta
21601 actggagtgc caaacaacat gcttcattct cctaaagtcc agcccaccct gtgtgacaat
21661 caaaaagcac tctaccattt tctcaatacc cattcgcctt attttcgctc tcatcgtaca
21721 cacatcgaaa gggccactgc gttcgaccgt atggatgtgc aataatgatt catgtaaaca
21781 acgtgttcaa taaacagcac tttatttttt acatgtatcg aggctctgga ttacttattt
21841 atttacaagt cgaatgggtt ctgacgagaa tcagaatgac ccgcaggcag tgatacgttg
21901 cggaactgat acttgggttg ccacttgaat tcgggaatca ccaacttggg aaccggtata
21961 tcgggcagga tgtcactcca cagctttctg gtcagctgca aagctcccag caggtcagga
22021 gccgaaatct tgaaatcaca attaggacca gtgctctgag cgcgagagtt gcggtacacc
22081 ggattgcagc actgaaacac catcagcgac ggatgtctta cgcttgccag cacggtggga
22141 tctgcaatca tgcccacatc cagatcttca gcattggcaa tgctgaacgg ggtcatcttg
22201 caggtctgcc tacccatggc gggcacccaa ttaggcttgt ggttacaatc gcagtgcagg
22261 gggatcagta tcatcttggc ctgatcctgt ctgattcctg gatacacggc tctcatgaaa
22321 gcatcatatt gcttgaaagc ctgctgggct ttactaccct cggtataaaa catcccgcag
22381 gacctgctcg aaaactggtt agctgcgcag ccggcatcat tcacacagca gcgggcgtca
22441 ttgttggcta tttgcaccac acttctgccc cagcggtttt gggtgatttt ggttcgctcg
22501 ggattctcct tcaaggctcg ttgtccgttc tcgctggcca catccatctc gataatctgc
22561 tccttctgaa tcataatatt gccatgcaag cacttcagct tgccctcata atcattgcag
22621 ccatgaggcc acaacgcaca gcctgtacat tcccaattat ggtgggcgat ctgagaaaaa
22681 gaatgtatca ttccctgcag aaatcttccc atcatcgtgc tcagtgtctt gtgactagtg
22741 aaagttaact ggatgcctcg gtgctcctcg ttcacgtact ggtgacagat gcgcttgtat
22801 tgttcgtgct gctcaggcat tagtttaaaa gaggttctaa gttcgttatc cagcctgtac
22861 ttctccatca gcagacacat cacttccatg cctttctccc aagcagacac caggggcaag
22921 ctaatcggat tcttaacagt gcaggcagca gctcctttag ccagagggtc atctttggcg
22981 atcttctcaa tgcttctttt gccatccttc tcaacgatgc gcacgggcgg gtagctgaaa
23041 cccactgcta caagttgcgc ctcttctctt tcttcttcgc tgtcttgact gatgtcttgc
23101 atggggacat gtttggtctt ccttggcttc tttttcgggg gtatcggagg aggaggactg
23161 tcgctccgtt ccggagacag ggaggattgt gacgtttcgc tcaccattac caactgactg
23221 tcggtagaag aacctgaccc cacacggcga caggtgtttc tcttcggggg cagaggtgga
23281 ggcgattgcg aagggctgcg gtccgacctg gaaggcggat gactggcaga acccttccg
23341 cgttcggggg tgtgctccct gtggcggtcg cttaactgat ttccttcgcg gctggccatt
```

FIG. 28A-6

```
23401 gtgttctcct aggcagagaa acaacagaca tggaaactca gccattgctg tcaacatcgc
23461 cacgagtgcc atcacatctc gtcctcagcg acgaggaaaa ggagcagagc ttaagcattc
23521 caccgcccag tcctgccacc acctctaccc tagaagataa ggaggtcgac gcatctcatg
23581 acatgcagaa taaaaaagcg aaagagtctg agccagacat cgaacaagac ccgggctatg
23641 tgacaccggt ggaacacgag gaagagttga aacgctttct agagagagag gatgaaaact
23701 gcccaaaaca gcaagcggat aactatcacc aagatgctgg aaatagggat cagaacaccg
23761 actacctcat agggcttgac ggggaagacg cgctccttaa acatctagca agacagtcac
23821 tcatagtcaa ggatgcatta ttggacagaa ctgaagtgcc catcagtgtc gaagagctca
23881 gccgcgccta cgagcttaac ctattttcac ctcgtactcc ccccaaacgt cagccaaacg
23941 gcacctgcga gccaaatcct cgcttaaact tttatccagc ttttgctgtg ccagaagtac
24001 tggctaccta tcacatcttt tttaaaaatc aaaaaattcc agtctcctgc cgcgctaatc
24061 gcacccgcgc cgatgcccta ctcaatctgg gacctggttc acgcttacct gatatagctt
24121 ccttggaaga ggttccaaag atcttcgagg gtctgggcaa taatgagact cgggccgcaa
24181 atgctctgca aaagggagaa aatggcatgg atgagcatca cagcgttctg gtggaattgg
24241 aaggcgataa tgccagactc gcagtactca agcgaagcgt cgaggtcaca cactttgcat
24301 accccgctgt caacctgccc cctaaagtca tgacggccgt catggaccag ttactcatta
24361 agcgcgcaag tccccttttca gaagacatgc atgacccaga tgcctgtgat gagggtaaac
24421 cagtggtcag tgatgagcag ctaacccgat ggctgggcac cgactctccc cgggatttgg
24481 aagagcgtcg caagcttatg atggccgtgg tgctggttac cgtagaacta gagtgtcttc
24541 ggcgtttctt taccgattca gaaaccttgc gcaaactcga agagaatctg cactacactt
24601 ttagacacgg ctttgtgcgg caggcatgca agatatctaa cgtggaactc accaacctgg
24661 tttcctacat gggtattctg catgagaatc gcctaggaca aagcgtgctg cacagcaccc
24721 ttaaggggga agcccgccgt gattacatcc gcgattgtgt ttatctctac ctgtgccaca
24781 cgtggcaaac cggcatgggt gtatggcagc aatgtttaga agaacagaac ctgaaagagc
24841 taaacaagct cttacagaaa tctcttaagg ttctgtggac agggttcgac gagcgcaccg
24901 tcgcttccga cctggcagac ctcatcttcc cagagcgtct cagggttact ttgcgaaacg
24961 gactgcctga ctttatgagc cagagcatgc ttaacaattt tcgctctttc atcctggaac
25021 gctccggtat cctgcccgcc acctgctgcg cactgccctc cgactttgtg cctctcacct
25081 accgcgaatg ccccccgccg ctatggagtc actgctacct gttccgtctg gccaactacc
25141 tctcctacca ctcggatgtg atcgaggatg tgagcggaga cggcttgctg gagtgtcact
25201 gccgctgcaa tctgtgcacg ccccaccggt ccctagcttg caacccccag ttgatgagcg
25261 aaacccagat aataggcacc tttgaattgc aaggccccag cagccaaggc gatgggtctt
25321 ctcctgggca aagtttaaaa ctgaccccgg gactgtggac ctccgcctac ttgcgcaagt
25381 ttgccccgga agattaccac ccctatgaaa tcaagttcta tgaggaccaa tcacagcctc
25441 cgaaagccga actttcggcc tgcgtcatca cccaggggc aattctggcc caattgcaag
25501 ccatccaaaa atcccgccaa gaatttctac tgaaaaaggg taaggggtc taccttgacc
25561 cccagaccgg cgaggaactc aacacaaggt tccctcagga tgtcccaacg acgagaaagc
25621 aagaagttga aggtgcagcc gccgccccca gaagatatgg aggaagattg ggacagtcag
25681 gcagaggaag cggaggagga ggacagtctg gaggacagtc tggaggaaga cagtttggag
25741 gaggaaaacg aggaggcaga ggaggtggaa gaagtaaccg ccgacaaaca gttatcctcg
25801 gctgcggaga caagcaacag cgctaccatc tccgctccga gtcgaggaac ccggcggcgt
25861 cccagcagta gatgggacga gaccggacgc ttcccgaacc caaccagcgc ttccaagacc
25921 ggtaagaagg atcggcaggg atacaagtcc tggcgggggc ataagaatgc catcatctcc
25981 tgcttgcatg agtgcggggg caacatatcc ttcacgcggc gctacttgct attccatcat
26041 ggggtgaact ttccgcgcaa tgttttgcat tactaccgtc acctccacag cccctactat
26101 agccagcaaa tcccggcagt ctcgacagat aaagacagcg gcggcgacct ccaacagaaa
26161 accagcagcg gcagttagaa aatacacaac aagtgcagca acaggaggat taaagattac
26221 agccaacgag ccagcgcaaa cccgagagtt aagaaatcgg atctttccaa ccctgtatgc
26281 catcttccag cagagtcggg gccaagagca ggaactgaaa ataaaaaacc gatctctgcg
26341 ttcgctcacc agaagttgtt tgtatcacaa gagcgaagat caacttcagc gcactctcga
26401 ggacgccgag gctctcttca acaagtactg cgcgctgact cttaaagagt aggcagcgac
26461 cgcgcttatt caaaaaaggc gggaattaca tcatcctcga catgagtaaa gaaattccca
26521 cgccttacat gtggagttat cagccccaaa tgggattggc ggcaggcgcc tcccaggact
26581 actccacccg catgaattgg ctcagcgccg ggccttctat gatttctcga gttaatgata
26641 tacgcgccta ccgaaaccaa atacttttgg aacagtcagc tcttaccacc acgccccgcc
26701 aacaccttaa tcccagaaat tggcccgccg ccctagtgta ccaggaaagt cccgctccca
26761 ccactgtatt acttcctcga gacgcccagg ccgaagtcca aatgactaat gcaggtgcgc
26821 agttagcggg cggctccacc ctatgtcgtc acaggcctcg gcataatata aaacgcctga
26881 tgatcagagg ccgaggtatc cagctcaacg acgagtcggt gagctctccg cttggtctac
26941 gaccagacgg aatctttcag attgccggct gcgggagatc ttccttcacc cctcgtcagg
27001 ctgttctgac tttggaaagt tcgtcttcgc aaccccgctc gggcggaatc gggaccgttc
27061 aatttgtgga ggagtttact ccctctgtct acttcaaccc cttctccgga tctcctgggc
27121 actacccgga cgagttcata ccgaacttcg acgcgattag cgagtcagtg gacggctacg
27181 attgatgtct ggtgacgcgg ctgagctatc tcggctgcga catctagacc actgccgccg
27241 ctttcgctgc tttgcccggg aactcattga gttcatctac ttcgaactcc ccaaggatca
```

FIG. 28A-7

```
27301 ccctcaaggt ccggcccacg gagtgcggat tactatcgaa ggcaaaatac actctcgcct
27361 gcaacgaatt ttctcccagc ggcccgtgct gatcgagcga gaccagggaa acaccacggt
27421 ttccatctac tgcatttgta atcaccccgg attgcatgaa agcctttgct gtcttatgtg
27481 tactgagttt aataaaaact gaattaagac tctcctacgg actgccgctt cttcaacccg
27541 gattttacaa ccagaagaac gaaacttttc ctgtcgtcca ggactctgtt aacttcacct
27601 ttcctactca caaactagaa gctcaacgac tacaccgctt ttccagaagc attttcccta
27661 ctaatactac tttcaaaacc ggaggtgagc tccaaggtct tcctacagaa aacccttggg
27721 tggaagcggg ccttgtagtg ctaggaattc ttgcgggtgg gcttgtgatt attctttgct
27781 acctatacac accttgcttc actttcctag tggtgttgtg gtattggttt aaaaaatggg
27841 gcccatacta gtcttgcttg ttttactttc gcttttggaa ccgggttctg ccaattacga
27901 tccatgtcta gacttcgacc cagaaaactg cacacttact tttgcacccg acacaagccg
27961 catctgtgga gttcttatta agtgcggatg ggaatgcagg tccgttgaaa ttacacacaa
28021 taacaaaacc tggaacaata ccttatccac cacatgggag ccaggagttc ccgagtggta
28081 cactgtctct gtccgaggtc ctgacggttc catccgcatt agtaacaaca ctttcatttt
28141 ttctgaaatg tgcgatctgg ccatgttcat gagcaaacag tattctctat ggcctcctag
28201 caaggacaac atcgtaacgt tctccattgc ttattgcttg tgcgcttgcc ttcttactgc
28261 tttactgtgc gtatgcatac acctgcttgt aaccactcgc atcaaaaacg ccaataacaa
28321 agaaaaaatg ccttaacctc tttctgttta cagacatggc ttctcttaca tctctcatat
28381 ttgtcagcat tgtcactgcc gctcacggac aaacagtcgt ctctatccct ctaggacata
28441 attacactct cataggaccc ccaatcactt cagaggtcat ctggaccaaa ctgggaagcg
28501 ttgattactt tgatataatc tgcaacaaaa caaaaccaat aatagtaact tgcaacatac
28561 aaaatcttac attgattaat gttagcaaag tttacagcgg ttactattat ggttatgaca
28621 gatacagtag tcaatataga aattacttgg ttcgtgttac ccagttaaaa accacgaaaa
28681 tgccaaatat ggcaaagatt cgatccgatg acaattctct agaaactttt acatctccca
28741 ccacacccga cgaaaaaaac atcccagatt caatgattgc aattgttgca gcggtggcag
28801 tggtgatggc actaataata atatgcatgc ttttatatgc ttgtcgctac aaaaagtttc
28861 atcctaaaaa acaagatctc ctactaaggc ttaacattta atttcttttt atacagccat
28921 ggtttccact accacattcc ttatgcttac tagtcttgca actctgactt ctgctcgctc
28981 acacctcact gtaactatag gctcaaactg cacactaaaa ggacctcaag gtggtcatgt
29041 cttttggtgg agaatatatg acaatggatg gtttacaaaa ccatgtgacc aacctggtag
29101 atttttctgc aacggcagag acctaaccat tatcaacgtg acagcaaatg acaaaggctt
29161 ctattatgga accgactata aaagtagttt agattataac attattgtac tgccatctac
29221 cactccagca ccccgcacaa ctactttctc tagcagcagt gtcgctaaca atacaatttc
29281 caatccaacc tttgccgcgc ttttaaaacg cactgtgaat aattctacaa cttcacatac
29341 aacaatttcc acttcaacaa tcagcattat cgctgcagtg acaattggaa tatctattct
29401 tgtttttacc ataacctact acgcctgctg ctatagaaaa gacaaacata aaggtgatcc
29461 attacttaga tttgatattt aatttgttct ttttttttt atttacagta tggtgaacac
29521 caatcatggt acctagaaat ttcttcttca ccatactcat ttgtgcattt aatgtttgcg
29581 ctactttcac agcagtagcc acagcaaccc cagactgtat aggagcattt gcttcctatg
29641 cacttttgc ttttgttact tgcatctgcg tatgtagcat agtctgcctg gttattaatt
29701 ttttccaact tctagactgg atccttgtgc gaattgccta cctgcgccac catcccgaat
29761 accgcaacca aaatatcgcg gcacttctta gactcatcta aaaccatgca ggctatacta
29821 ccaatatttt tgcttctatt gcttccctac gctgtctcaa ccccagctgc ctatagtact
29881 ccaccagaac accttagaaa atgcaaattc caacaaccgt ggtcatttct tgcttgctat
29941 cgagaaaaat cagaaattcc cccaaattta ataatgattg ctggaataat taatataatc
30001 tgttgcacca taatttcatt tttgatatac cccctatttg attttggctg gaatgctccc
30061 aatgcacatg atcatccaca agacccagag gaacacattc ccctacaaaa catgcaacat
30121 ccaatagcgc taatagatta cgaaagtgaa ccacaacccc cactactccc tgctattagt
30181 tacttcaacc taaccggcgg agatgactga aacactcacc acctccaatt ccgccgagga
30241 tctgctcgat atggacggcc gcgtctcaga acagcgactt gcccaactac gcatccgcca
30301 gcagcaggaa cgcgcggcca aagagctcag agatgtcatc caaattcacc aatgcaaaaa
30361 aggcatattc tgtttggtaa aacaagccaa gatatcctac gagatcaccg ctactgacca
30421 tcgcctctct tacgaacttg gcccccaacg acaaaaattt acctgcatgg tgggaatcaa
30481 ccccatagtt atcacccagc aaagtggaga tactaagggt tgcattcact gctcctgcga
30541 ttccatcgag tgcacctaca ccctgctgaa gaccctatgc ggcctaagag acctgctacc
30601 aatgaattaa aaaatgatta ataaaaaatc acttacttga aatcagcaat aaggtctctg
30661 ttgaaatttt ctcccagcag cacctcactt ccctcttccc aactctggta ttctaaaccc
30721 cgttcagcgg catactttct ccatacttta aaggggatgt caaattttag ctcctctcct
30781 gtacccacaa tcttcatgtc tttcttccca gatgaccaag agagtccggc tcagtgactc
30841 cttcaaccct gtctacccct atgaagatga aagcacctcc caacacccct ttataaaccc
30901 agggtttatt tccccaaatg gcttcacaca aagcccagac ggagttctta ctttaaaatg
30961 tttaacccca ctaacaacca caggcggatc tctacagcta aaagtgggag ggggacttac
31021 agtggatgac actgatggta ccttacaaga aaacatacgt gctacagcac ccattactaa
31081 aaataatcac tctgtagaac tatccattgg aaatggatta gaaactcaaa acaataaact
31141 atgtgccaaa ttgggaaatg ggttaaaatt taacaacggt gacatttgta taaaggatag
```

FIG. 28A-8

```
31201 tattaacacc ttatggactg gaataaaccc tccacctaac tgtcaaattg tggaaaacac
31261 taatacaaat gatggcaaac ttactttagt attagtaaaa aacggagggc ttgttaatgg
31321 ctacgtgtct ctagttggtg tatcagacac tgtgaaccaa atgttcacac aaaagacagc
31381 aaacatccaa ttaagattat attttgactc ttctggaaat ctattaactg atgaatcaga
31441 cttaaaaatt ccacttaaaa ataaatcttc tacagcgacc agtgaaactg tagccagcag
31501 caaagccttt atgccaagta ctacagctta tcccttcaac accactacta gggatagtga
31561 aaactacatt catggaatat gttactacat gactagttat gatagaagtc tatttccctt
31621 gaacatttct ataatgctaa acagccgtat gatttcttcc aatgttgcct atgccataca
31681 atttgaatgg aatctaaatg caagtgaatc tccagaaagc aacatagcta cgctgaccac
31741 atccccctttt ttcttttctt acattacaga agacgacaac taaaataaag tttaagtgtt
31801 tttatttaaa atcacaaaat tcgagtagtt attttgcctc caccttccca tttgacagaa
31861 tacaccaatc tctccccacg cacagcttta aacatttgga taccattaga gatagacatt
31921 gttttagatt ccacattcca aacagtttca gagcgagcca atctggggtc agtgatagat
31981 aaaaatccat cgcgatagtc ttttaaagcg ctttcacagt ccaactgctg cggatgcgaa
32041 tccggagtct ggatcacggt catctggaag aagaacgatg ggaatcataa tccgaaaacg
32101 gtatcggacg attgtgtctc atcaaaccca caagcagccg ctgtctgcgt cgctccgtgc
32161 aactgctgtt tatgggatca gggtccacag tgtcctgaag catgatttta atagcccttа
32221 acatcaactt tctggtgcga tgcgcgcagc aacgcattct gatttcactc aaatctttgc
32281 agtaggtaca acacattatt acaatattgt ttaataaacc ataattaaaa gcgctccagc
32341 caaaactcat atctgatata atcgcccctg catgaccatc ataccaaagt ttaatataaa
32401 ttaaatgacg ttccctcaaa aacacactac ccacatacat gatctctttt ggcatgtgca
32461 tattaacaat ctgtctgtac catggacaac gttggttaat catgcaaccc aatataacct
32521 tccggaacca cactgccaac accgctcccc cagccatgca ttgaagtgaa ccctgctgat
32581 tacaatgaca atgaagaacc caattctctc gaccgtgaat cacttgagaa tgaaaaatat
32641 ctatagtggc acaacataga cataaatgca tgcatcttct cataattttt aactcctcag
32701 gatttagaaa catatcccag ggaataggaa gctcttgcag aacagtaaag ctggcagaac
32761 aaggaagacc acgaacacaa cttacactat gcatagtcat agtatcacaa tctggcaaca
32821 gcgggtggtc ttcagtcata gaagctcggg tttcattttc ctcacaacgt ggtaactggg
32881 ctctggtgta agggtgatgt ctggcgcatg atgtcgagcg tgcgcgcaac cttgtcataa
32941 tggagttgct tcctgacatt ctcgtatttt gtatagcaaa acgcggccct ggcagaacac
33001 actcttcttc gccttctatc ctgccgctta gcgtgttccg tgtgatagtt caagtacagc
33061 cacactctta agttggtcaa aagaatgctg gcttcagttg taatcaaaac tccatcgcat
33121 ctaattgttc tgaggaaatc atccacggta gcatatgcaa atcccaacca agcaatgcaa
33181 ctggattgcg tttcaagcag gagaggagag ggaagagacg gaagaaccat gttaattttt
33241 attccaaacg atctcgcagt acttcaaatt gtagatcgcg cagatggcat ctctcgcccc
33301 cactgtgttg gtgaaaaagc acagctaaat caaaagaaat gcgattttca aggtgctcaa
33361 cggtggcttc caacaaagcc tccacgcgca catccaagaa caaaagaata ccaaaagaag
33421 gagcattttc taactcctca atcatcatat tacattcctg caccattccc agataatttt
33481 cagctttcca gccttgaatt attcgtgtca gttcttgtgg taaatccaat ccacacatta
33541 caaacaggtc ccggagggcg ccctccacca ccattcttaa acacaccctc ataatgacaa
33601 aatatcttgc tcctgtgtca cctgtagcga attgagaatg gcaacatcaa ttgacatgcc
33661 cttggctcta agttcttctt taagttctag ttgtaaaaac tctctcatat tatcaccaaa
33721 ctgcttagcc agaagccccc cgggaacaag agcaggggac gctacagtgc agtacaagcg
33781 cagacctccc caattggctc cagcaaaaac aagattggaa taagcatatt gggaaccgcc
33841 agtaatatca tcgaagttgc tggaaatata atcaggcaga gtttcttgta aaaattgaat
33901 aaaagaaaaa tttgccaaaa aaacattcaa aacctctggg atgcaaatgc aataggttac
33961 cgcgctgcgc tccaacattg ttagttttga attagtctgc aaaaataaaa aaaaaaacaa
34021 gcgtcatatc atagtagcct gacgaacagg tggataaatc agtctttcca tcacaagaca
34081 agccacaggg tctccagctc gaccctcgta aaacctgtca tggtgattaa acaacagcac
34141 cgaaagttcc tcgcggtgac cagcatgaat aattcttgat gaagcataca atccagacat
34201 gttagcatca gttaacgaga aaaaacagcc aacatagcct ttgggtataa ttatgcttaa
34261 tcgtaagtat agcaaagcca cccctcgcgg atacaaagta aaaggcacag gagaataaaa
34321 aatataatta tttctctgct gctgttcagg caacgtcgcc cccggtccct ctaaatacac
34381 atacaaagcc tcatcagcca tggcttacca gacaaagtac agcgggcacg cacaagctct
34441 aaagtcactc tccaacctct ccacaatata tatacacaag ccctaaactg acgtaatggg
34501 agtaaagtgt aaaaaatccc gccaaaccca acacacaccc cgaaactgcg tcaccaggga
34561 aaagtacagt ttcacttccg caatcccaac aagcgtcact tcctctttct cacggtacgt
34621 cacatcccat taacttgcaa cgtcattttc ccacggccgc gccgccccgt ttagccgtta
34681 accccacagc caatcaccac acaccccaca atttttaaaa tcacctcatt tacatattgg
34741 caccattcca tctataaggt atattattga tgatg
```

SEQ ID NO: 12

FIG. 28A-9

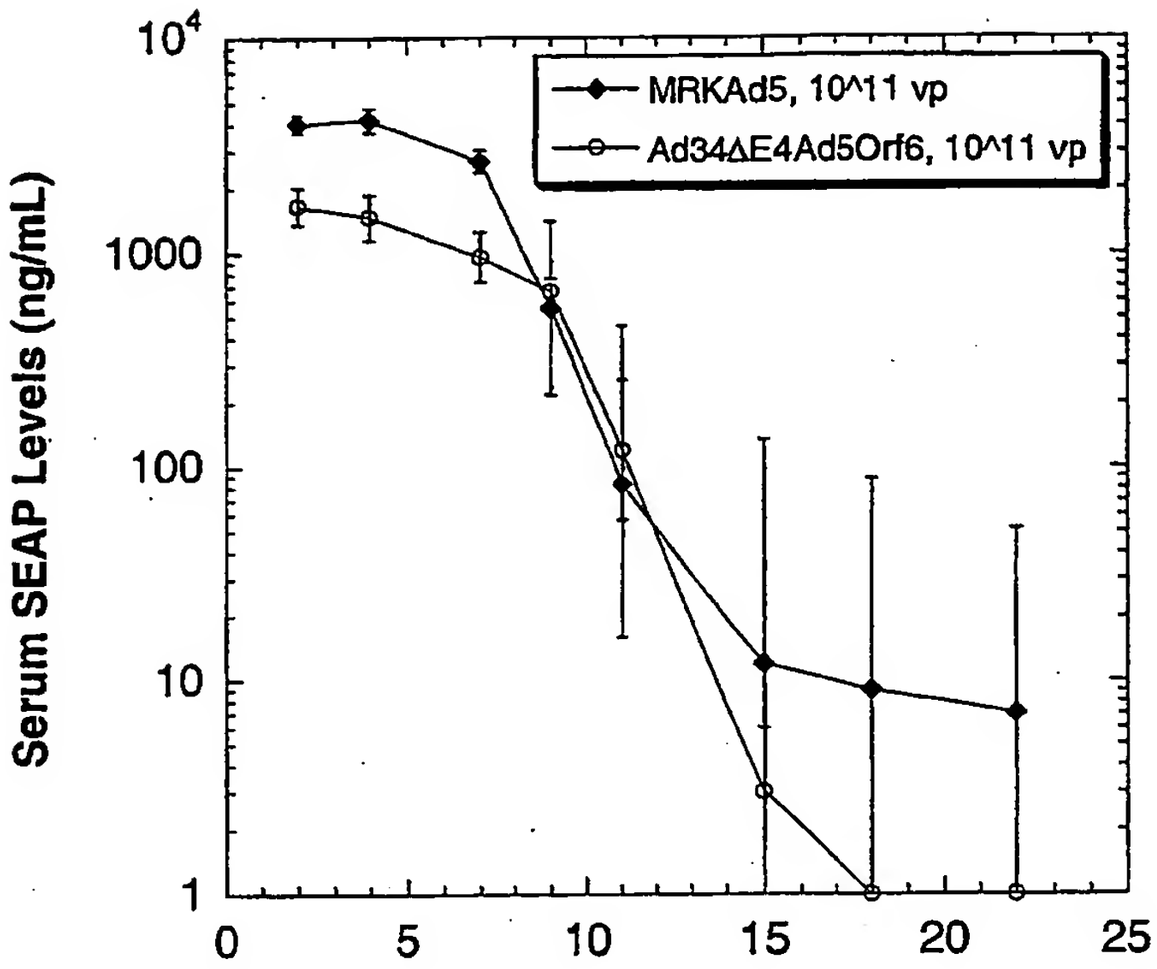

MRKAd5, 10^11 vp
Ad34ΔE4Ad5Orf6, 10^11 vp
Serum SEAP Levels (ng/mL)
10^4
1000
100
10
1
0
5
10
15
20
25

FIG. 29

Vaccine Wk 0, 4, 24	Monkey ID	Pre		Wk 4		Wk 8		Wk 24		Wk 28		Wk 36	
		Mock	Gag[a]	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
MRKAd5gag, 10^11 vp	00C018	1	5	13	1025	0	824	9	756	0	474	0	383
MRKAd5gag, 10^11 vp	00C034	0	4	5	219	5	404	3	445	3	339	0	216
MRKAd5gag, 10^11 vp	00C058	4	4	3	1086	0	440	4	1439	0	2338	0	940
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	00D038	6	8	5	111	1	301	0	224	1	535	0	233
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	00D042	6	30	4	89	4	264	1	73	0	181	0	69
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	00D066	3	18	1	118	1	816	0	429	0	439	0	273

FIG. 30

Vaccine	Monk ID	IFN-γ+CD4+CD3+ per 10^6 Lymphocytes		IFN-γ+CD8+CD3+ per 10^6 Lymphocytes	
		Mock	Gag[a]	Mock	Gag[a]
Ad34ΔE1gagΔE4Ad5Orf6	00D038	22	154	130	450
	00D042	32	118	96	171
	00D066	12	238	150	442

FIG. 31

Vaccine T=0, 4 wks	Vaccine T=24 wks	Monkey ID	Pre		T=4 wks		T=8 wks		T=24 wks		T=28 wks		T=32 wks	
			Mock	Gag[a]	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag	Mock	Gag
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10^10 vp	00D018	4	6	1	84	5	334	5	99	0	308	3	244
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10^10 vp	00D044	1	1	8	79	0	374	8	136	0	493	1	253
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10^10 vp	00D064	4	6	1	125	8	655	6	145	0	351	1	236
Naïve		00D087	1	1	3	3	8	54	6	8	5	5	3	0

FIG. 32

Vaccine (T=0, 4 Wks)	Vaccine (T=24 Wk)	Monkey ID	IFN-γ+CD4+CD3+ per 10^6 Lymphocytes		IFN-γ+CD8+CD3+ per 10^6 Lymphocytes	
			Mock	Gag	Mock	Gag
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10^10 vp	00D016	62	433	176	1288
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10^10 vp	00D044	136	593	323	1871
Ad34ΔE1gagΔE4Ad5Orf6, 10^11 vp	Ad35ΔE1gagΔE4Ad5Orf6, 10^10 vp	00D064	188	785	292	992

FIG. 33